ELSEVIER

Toxicology Letters 110 (1999) 145–175

Toxicology Letters

www.elsevier.com/locate/toxlet

Methylglyoxal in living organisms Chemistry, biochemistry, toxicology and biological implications

Miklós Péter Kalapos *

Theoretical Biology Research Group, H-1029, Budapest, Dámvad utca 18, Hungary

Received 7 June 1999; received in revised form 28 July 1999; accepted 23 August 1999

Abstract

Despite the growing interest towards methylglyoxal and glyoxalases their real role in metabolic network is still obscure. In the light of developments several reviews have been published in this field mainly dealing with only a narrow segment of this research area. In this article a trial is made to present a comprehensive overview of methylglyoxal research, extending discussion from chemistry to biological implications by reviewing some important characteristics of methylglyoxal metabolism and toxicity in a wide variety of species, and emphasizing the action of methylglyoxal on energy production, free radical generation and cell killing. Special attention is paid to the discussion of α-oxoaldehyde production in the environment as a potential risk factor and to the possible role of this α-dicarbonyl in diseases. Concerning the interaction of methylglyoxal with biological macromolecules (DNA, RNA, proteins) an earlier review (Kalapos, Toxicology Letters, 73, 1994, 3–24) means a supplementation to this paper, thus hoping the avoidance of unnecessary bombast. The paper arrives at the conclusion that since the early stage of evolution the function of methylglyoxalase pathway has been related to carbohydrate metabolism, but its significance has been changed over the thousands of years. Namely, at the beginning of evolution methylglyoxalase path was essential for the reductive citric acid cycle as an anaplerotic route, while in the extant metabolism it concerns with the detoxification of methylglyoxal and plays some regulatory role in triose-phosphate household. As there is a tight junction between methylglyoxal and carbohydrate metabolism its pathological role in the events of the development of diabetic complications emerges in a natural manner and further progress is hoped in this field. In contrast, significant advancement cannot be expected in relation to cancer research

Abbreviations: 2, 4-dinitrophenylhydrazine (DNPH); Inorganic phosphate (P_i); Semicarbazide-sensitive amine oxidase (SSAO); Periodo-acid Schiff reaction (PAS); Reduced glutathione (GSH); Ornithine decarboxylase (OCD); γ-Glutamyl transpeptidase (γ-GT); Glutathione S-transferase placental form (GST-P); Dose for 50% incidence of skin tumours (ID_{50}); Clastogenic ability (CD_{20}); *N*-metil-*N*′-nitro-*N*-nitrosoguanidine (MNNG); 2-acetylamino-fluorene (2-AAF); Cyclic-adenosine monophosphate (cAMP); Intracellular potassium concentration ($[K^+]_{IC}$); Isopropylthiogalactoside (IPTG); Potassium efflux system in *E. coli* (Kef); High affinity K^+ uptake system in *E. coli* (Kdp); Heparin-binding epidermal growth factor (HB-EGF); Insulin dependent diabetes mellitus (IDDM); Non-insulin dependent diabetes mellitus (NIDDM).

* Tel./fax: + 36-1-3975225.

PII: S0378-4274(99)00160-5

Keywords: Methylglyoxal; Glyoxalases; Free radicals; Carcinogenesis; Diabetes mellitus; Gluconeogenesis

1. Introduction

Methylglyoxal research most probably started in the last decades of 19th century. At that time the synthesis, the chemical characterization and reactions of methylglyoxal were in the centre of investigation (Baumann, 1885; von Pechmann, 1887). However, in 1913 an enzyme system, believed to be only one enzyme at the time of its discovery and named glyoxalase, was described and thought to be capable of converting α-oxoaldehydes into their α-hydroxy-carboxylic acid counterparts (Dakin and Dudley, 1913a; Neuberg, 1913). Methylglyoxal was also a substrate of this newly discovered enzyme. Here started the biochemical research on methylglyoxal.

Till the full development of the Embden–Meyerhof scheme of glucose breakdown between 1932 and 1939, methylglyoxal was suggested to lie in the main stream of glycolysis leading to lactate formation (Bernhauer and Görlich, 1929). Only after more than two decades of sagging interest in this field was it suggested by Szent-Györgyi in the 1960s that methylglyoxal as retine and glyoxalase I as promine may have had a crucial regulatory function in cell division (Szent-Györgyi, 1965). The theory of Szent-Györgyi gave a sudden impulse to research. At the end of 1970s and in the first half of 1980s the experimental work led to the discovery of several enzymes taking place in methylglyoxal metabolism (Cooper, 1984). And also, the cancerostatic action of the aldehyde was extensively investigated (Szent-Györgyi, 1973). Despite the endeavours the real role of methylglyoxal and also of glyoxalases remained obscure whereas several theories were worked out (Szent-Györgyi, 1965; Gillespie, 1981). But no convincing evidence for any of the roles is so far available (Kalapos, 1999a).

Methylglyoxal has recently been brought into the focus of interest in the account of its ability to result in pathological events happening in diabetes mellitus. In the light of many new developments and space limitation to this paper, only then can meaningful contribution be made if in some cases, when it is appropriate, the reader is referred to original publications as well as review papers in which further detailed accounts and experimental evidence can be found. The specific purpose of this article is to examine some important characteristics of methylglyoxal metabolism and toxicity. Therefore, in this review the discussion is limited to the chemistry of methylglyoxal, to the studies of methylglyoxal metabolism in different species, to in vivo effects of methylglyoxal as well as to in vitro actions of it, particularly emphasizing its action on energy production, free radical generation and cell killing, and finally to the possible role of this α-dicarbonyl in diseases.

2. Chemistry of methylglyoxal

Methylglyoxal (pyruvaldehyde, pyruvic aldehyde, 2-oxopropanal, 2-ketoproprion-aldehyde, acetyl-formaldehyde) is a yellow liquid with characteristic pungent odor.

2.1. Chemical preparation

Maybe the first method for the preparation of methylglyoxal was described by von Pechmann (1887). As to the method, by warming isonitrosoacetone in the presence of diluted sulfuric acid methylglyoxal was obtained (von Pechmann, 1887). Dihydroxyacetone can also be converted into methylglyoxal in such a way that dihydroxyacetone and aluminium sulfate are dissolved in sulfuric acid, and then the mixture is heated in distilling flask (Patterson and Lazarow, 1955). However, the synthesis of methylglyoxal by either of the above mentioned procedures results in the formation of several contaminants, which are as follows: glycols, formaldehyde and formate (Brum, 1966). Methylglyoxal may be prepared

from methylglyoxal dimethylacetal (1,1-dimethoxypropanone) by acid hydrolysis, too (Kellum et al., 1978). And also, the oxidation of acetone with selenium dioxide followed by distillation under nitrogen atmosphere yields methylglyoxal (Brum, 1966; Paulmier, 1986; Clelland and Thornalley, 1990). This method has been used for the synthesis of ^{14}C labeled methylglyoxal, as well (Brum, 1966; Clelland and Thornalley, 1990). Both 2-^{14}C-methylglyoxal and 1,3-^{14}C-methylglyoxal may be obtained by this procedure and the product is free of those impurities detected when either dihydroxyacetone or isonitrosoacetone were used as starting materials for synthesis (Brum, 1966; Clelland and Thornalley, 1990).

It has been published that commercially available methylglyoxal is mainly contaminated with formaldehyde, and also with other impurities such as pyruvate, lactate and formate (Pourmotabbed and Creighton, 1986; Rae et al., 1990). The amount of formaldehyde may account for as many as 30% in the manufactured methylglyoxal solution (Pourmotabbed and Creighton, 1986). All these make the purification of manufacturers' methylglyoxal necessary.

Essentially two methods have been described for the purification of methylglyoxal: ion exchange chromatography and distillation; latter has been either steam or vacum destillation. Dowex 1 ion exchange columns either in chloride or in bicarbonate form are able to remove only the acidic contaminants like lactate or pyruvate (Monder, 1967), therefore in the majority of cases the distillation of methylglyoxal under reduced pressure is chosen to purify the commercial material (Kermack and Matheson, 1957). Nevertheless, it has been reported that the destillate still contained impurities, e.g. formaldehyde and other trace elements (Rae et al., 1990). Probably for the above reason, sometimes distillation is followed by ion exchange column chromatography in order to clear away acid contamination retained after distillation (Griffith et al., 1983; Douglas et al., 1985). It has to be noticed that the acidic hydrolysis of dimethylacetal followed by distillation under reduced pressure is substantially free of impurities (Rae et al., 1990).

2.2. Determination of methylglyoxal

Over the decades several methods have been worked out to measure methylglyoxal levels both in vitro and in vivo systems. In the beginning mainly colorometric methods were developed and used for the determination of methylglyoxal: (i) the Ariyama's method using cyanide, arsenophospho-tungstinic acid under alkaline circumstances (Ariyama, 1928); (ii) the Friedemann's titration after oxidation performed by H_2O_2 (Friedemann, 1927); (iii) the semicarbazide method (Barrenscheen, 1928) and (iv) the reaction with 2,4-dinitrophenyl-hydrazine (DNPH) either followed by chromatographic separation of the resultant osazone (Barrenscheen and Braun, 1931; Barrenscheen and Dreguss, 1931; Fung and Grosjean, 1981), or coupled to further chemical reaction to eliminate the disturbing effects of other osazone derivatives (Johnson et al., 1962). The iodoform (Case and Cook, 1931) and the nitroprusside reactions (Barrenscheen and Braun, 1931) were employed only very rarely. The disadvantages of these methods are the lack of their specificity and the detection limit of those. A spectrophotometric assay for methylglyoxal using glyoxalase I was also applied (Racker, 1957).

In the last two decades several types of derivatization methods for methylglyoxal were worked out in a link to HPLC or gas chromatographic facilities. Basically, all the procedures use a derivatization of the α-oxoaldehyde with o-phenylenediamine or its derivatives like dichloro-1,2-phenylenediamine and 1,2-diamino-4,5-dimethoxybenzene detecting the amount of formed quinoxalines (Moree-Testa and Saint-Jalm, 1981; Ohmori et al., 1987a,b; McLellan et al., 1992). In the meantime, a gas chromatographic procedure without any derivatization of methylglyoxal has also been recommended (Peinado et al., 1987). Recently, the most refined technique for the assay of methylglyoxal is based on the above mentioned kind of derivatization (Chaplen et al., 1996). Using advanced methods the plasma concentration of this α-dicarbonyl is estimated to be in the order of 10 micromolar range.

2.3. Participation of methylglyoxal in chemical reactions

Investigations of the behaviour of methylglyoxal in water have been carried out. It has been revealed that methylglyoxal shows an enol-oxo tautomerization and the equilibrium is shifted towards the enol form by the increase of pH (Schauestein et al., 1980). In alkaline medium, methylglyoxal undergoes an intramolecular Cannizzaro rearrangement leading to lactate formation (Fedoronko and Königstein, 1969, 1971). In aqueous solution it is polymerized (de V. Moulds and Riley, 1938) and hydrated (Creighton et al., 1988). At pH 7 methylglyoxal persists in three forms and thermodynamically its monohydrate form is favoured (Creighton et al., 1988). The percentile distribution of monohydrate, dihydrate and unhydrated species is 56, 44% and trace amount, respectively (Thornalley, 1993). Similarly to other monosaccharides methylglyoxal also subjects to autoxidation, but the rate of its autoxidation is much lower than that of glyceraldehyde or dihydroxyaldehyde (Thornalley, 1985).

The photolysis of methylglyoxal resulting in radical production in the presence of molecular oxygen has been described (Atkinson et al., 1980). In addition, the radical formation from methylglyoxal may also be initiated by $OH^{\bullet}$, too (Atkinson et al., 1980).

The interaction of methylglyoxal with thiols and amines is well known, and has been discussed in details by several authors [for review see Schauestein et al. (1980)]. What here should be emphasized is that the aldehydic group is more susceptible to an attack by any other functional groups than the ketonic group (Leoncini, 1979). All in all, these reactions provide the chemical basis of the modification of amino acids, proteins and nucleic acids, and elucidate the mode of action of this α-oxoaldehyde in biological systems. Nevertheless, the discussion of the effect of methylglyoxal on macromolecules is avoided here as the problem has already been overviewed in details [for review see Kalapos (1994a)]. Some aspects of these reactions will be taken into account later while discussing the development of diabetic complications and cancer paradox.

Another part of methylglyoxal chemistry is its use in chemical syntheses of organic compounds. Particularly important is the so called Radziszewski synthesis during which methylglyoxal together with formaldehyde and ammonia lead to the formation of imidazole derivatives (Hofman, 1953). The possible evolutionary significance of this fact has recently been pointed out (Kalapos, 1998).

3. Methylglyoxal metabolism

3.1. Main pathways of methylglyoxal metabolism

The main routes of enzyme catalyzed methylglyoxal formation and utilization are well known, and have been reviewed in several papers (Leoncini, 1979; Cooper, 1984; Kimura and Inoue, 1993; Thornalley, 1993; Kalapos, 1994a). Thus, here only a briew overview of these pathways is given. The non-enzymatic formation of methylglyoxal having salient importance from toxicological point of view, is discussed in detail in Section 3.3.

3.1.1. Production of methylglyoxal

Methylglyoxal is synthesized by three kinds of enzymes: methylglyoxal synthase; cytochrome P450 IIE1 isozyme(s) and amine oxidase(s) participating in glycolytic bypass, acetone metabolism and amino acid breakdown, respectively (Fig. 1).

Methylglyoxal synthase (EC.4.2.99.11.) was first purified from *E(scherichia) coli* (Hopper and Cooper, 1971). Since then it has been found both in other procaryotic and in mammalian systems (Cooper, 1975; Ray and Ray, 1981). An interesting quality of this enzyme is the inhibition of its activity by inorganic phosphate (P_i) (Hopper and Cooper, 1971). This observation has led to the suggestion that the enzyme is regulated by P_i and in this way it plays a role in the control of glycolysis depending on the availability of intracellular P_i [for review see Cooper (1984)].

The possible role of cytochrome P450s (EC.1.14.14.1.) in acetone metabolism was recognized in 1980 (Coleman, 1980). The production of methylglyoxal, in monohydrated form, from ace-

tone needs the participation of isozymes belonging to the cytochrome P450 IIE1 gene subfamily (Casazza et al., 1984). The conversion of acetone into methylglyoxal happens in two consecutive steps via acetol as intermediate and consumes $NADPH + H^+$ (Casazza et al., 1984; Koop and Casazza, 1985). However, it should be noticed that isozymes belonging to other P450 gene subfamilies are also able to manage the transformation of acetol into pyruvaldehyde, though their share in the government of catalysis is significantly much less than that of cytochrome P450 IIE1 isozymes (Koop and Casazza, 1985). The most salient biochemical feature of this pathway is its inducibility. Its capacity can be induced by several agents (acetone, ethanol, pyrazole, imidazole, etc.) or under different physiolo-gical/pathological circumstances (e.g. in fasting or in diabetes mellitus) [for review see Gonzalez, (1989)].

Probably the investigation of methylglyoxal production from aminoacetone started in the 1950s and an amine oxidase was suggested to catalyze the conversion of aminoacetone to methylglyoxal (Elliott, 1960; Urata and Granick, 1963). Aminoacetone is an offshoot of threonine and glycine metabolism (Urata and Granick, 1963). Amine oxidases are known as a large family of enzymes in mammals and various members of the family are divided into classes depending on their cofactor requirement (Lyles, 1996). The

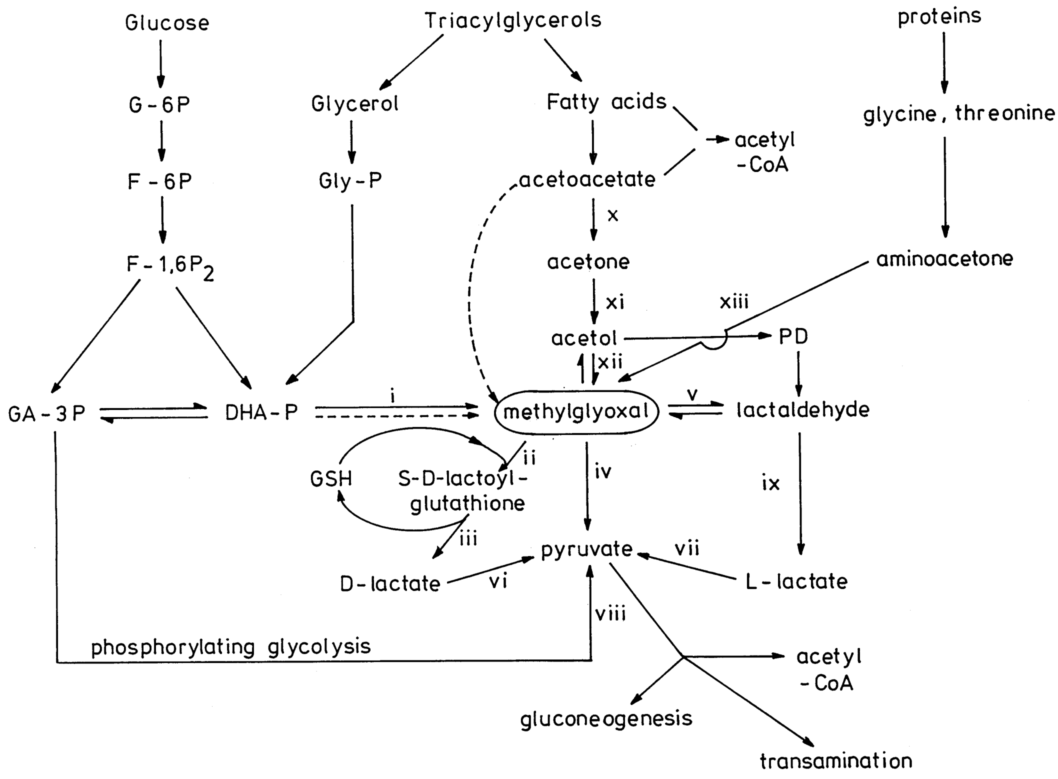

Fig. 1. Pathways of methylglyoxal metabolism. Abbreviations used in the figure: G-6P, glucose 6-phosphate; F-6P, fructose 6-phosphate; F-1,6P_2, fructose 1,6-bisphosphate; GA-3P, glyceraldehyde 3-phosphate; DHA-P, dihydroxyacetone-phosphate; Gly-P, glycerolphosphate; GSH, glutathione; PD, propanediol. The enzymes involved in the reactions: (i) methylglyoxal synthase; (ii) after a non-enzymatic reaction between methylglyoxal and glutathione glyoxalase I; (iii) glyoxalase II; (iv) α-oxoaldehyde dehydrogenase; (v) e.g. methylglyoxal reductase; (vi) D-lactate dehydrogenase; (vii) L-lactate dehydrogenase; (viii) the enzymes of phosphorylating glycolysis; (ix) e.g. lactaldehyde dehydrogenase; (x) acetoacetate decarboxylase and spontaneously, too; (xi) acetone monooxygenase; (xii) acetol monooxygenase; (xiii) amine oxidase. Cofactors are not shown in the flow-chart. Dotted lines represent the ways of non-enzymatic methylglyoxal formation. For details see the text.

amine oxidase of our interest, sometimes called aminoacetone oxidase, contains a cofactor having carbonyl(s) and hence being sensitive to semicarbazide inhibition (Lyles, 1996). However, there are other members of the semicarbazide-sensitive amine oxidase group being unable to metabolize aminoacetone, thus here the semicarbazide-sensitive amine oxidase (SSAO) (EC.1.4.3.6.), just like in the literature, is only referred to that enzyme possessing the ability to use benzylamine as a substrate (Lyles, 1996). SSAO exists in two forms, in a soluble form in the plasma and in a tissue-bound form in plasmalemmal membrane, and both are capable of converting aminoacetone into methylglyoxal (Lyles, 1996). SSAO is located only in some tissues and seems to be specific to mammals (for review see Lyles, 1996; Yu, 1998). Since aminoacetone appears to have the lowest K_M for SSAO, it is believed to be as potential physiological substrate (Lyles and Chalmers, 1992). The appearance of an amine oxidase in microbes has also been demonstrated, e.g. in *Staphylococcus aureus* (Elliott, 1959) and *Saccharomyces cerevisiae* (Inoue and Kimura, 1995). As yet it is not known whether such an activity is present in plants.

3.1.2. Degradation of methylglyoxal

The degradation of methylglyoxal mainly happens via glyoxalase system. The glyoxalase system consists of two enzymes and requires catalytic amount of reduced glutathione (GSH) (Racker, 1951). Glyoxalase I (S-lactoylglutathione methlylglyoxal lyase, EC.4.4.1.5.) acts on the adduct of methylglyoxal and glutathione, thus catalysing the one substrate isomeration of hemimercaptal into S-D-lactoylglutathione (for review see Vander Jagt, 1989; Mannervik and Ridderström, 1993). Subsequently, glyoxalase II (S-2-hydroxyacylglutathione hydrolase, EC.3.1.2.6.) splits S-D-lactoylglutathione into D-lactate and GSH (for review see Vander Jagt, 1989, 1993). Glyoxalase I is a metalloprotein, while glyoxalase II is not a metallohydrolase (Vander Jagt, 1989; Mannervik and Ridderström, 1993; Vander Jagt, 1993). In humans, glyoxalase I exhibits polymorphism as two alleles, GLO^1 and GLO^2, encode the enzyme (Vander Jagt, 1989). Three phenotypes of the enzyme, $GLO^{1\text{-}1}$, $GLO^{1\text{-}2}$, $GLO^{2\text{-}2}$ are known and identical kinetically (Vander Jagt, 1989). The phosphorylation as well as an induction of glyoxalase I have also been reported (Inoue et al., 1990; Principato et al., 1990).

The α-oxoaldehyde dehydrogenase [2-oxoaldehyde:NAD(P)$^+$ oxido-reductase, EC. 1.2.1.23.] is another enzyme engaged to methylglyoxal degradation (for review see Vander Jagt, 1982; Inoue and Kimura, 1995). First it was purified from sheep liver (Monder, 1965, 1967). Later on considerable work has been done in the field of its examination and the existence of enzyme in microorganisms has also been proven (Inoue and Kimura, 1995). Both NADP$^+$ and NAD$^+$ have been shown as coenzymes for the enzyme and the K_M value for methylglyoxal with respect to NADP$^+$ was smaller than with respect to NAD$^+$ (Monder, 1967). From plants this enzyme has not been reported yet.

3.2. Minor routes in methylglyoxal metabolism

Beside methylglyoxal synthase there are other glycolytic enzymes which take place in methylglyoxal metabolism either producing or utilizing it. It was 1978, when fructose 1,6-bisphosphate aldolase (EC.4.1.2.13) was reported to form a complex with pyruvaldehyde and orthophosphate (Grazi and Trombetta, 1978). During the backward reaction first dihydroxyacetone phosphate by interacting with the enzyme formed an aldolase-dihydroxyacetone phosphate complex which was then converted into pyruvaldehyde-aldolase complex in a reversible manner (Grazi and Trombetta, 1978). From the latter complex methylglyoxal was cleaved (Grazi and Trombetta, 1978). It is noticable that the mentioned pyruvaldehyde–aldolase intermediate was not detected when the enzyme functioned in the forward direction that is when it interacted with fructose 1,6-bisphosphate (Grazi and Trombetta, 1978). In the 1930s, it was already known that dihydroxyacetone phosphate condensed with a wide variety of aldehydes, among others with methylglyoxal, by aldol condensation in the presence of aldolase (Meyerhof, 1951). However, only in the 1990s was the reaction investigated in details showing the

formation of diketone phosphate from methylglyoxal and dihydroxyacetone phosphate (Rae et al., 1992). Interestingly, hydroxy-methylglyoxal, which otherwise with dihydroxyacetone phosphate would have yielded fructose 1-phosphate, did not prove an effective substrate (Rae et al., 1992). Considerable evidence have been gathered giving support to the release of methylglyoxal in the triose-phosphate isomerase (EC.5.3.1.1.) catalyzed reaction (Richard, 1985). Enediol intermediate of triose-phosphate interconversion is the source of methylglyoxal release (Richard, 1985; Knowles, 1991).

Since methylglyoxal contains two functional groups that may be either oxidized or reduced, it is not a surprise that the enzymes involved in oxido-reductions are capable of managing the conversion of methylglyoxal. Reduction of this α-oxoaldehyde is expected to result in two products, either acetol or lactaldehyde. Oxido-reductases and dehydrogenases are able to do this job and classified as families of these enzymes.

The family of oxido-reductases involves the enzymes usually represented as ALR1, ALR2 and ALR3, and called aldehyde reductase (alcohol:$NADP^+$ oxido-reductase, EC.1.1.1.2.), aldose reductase (alditol:$NADP^+$ oxido-reductase, EC.1.1.1.21.) and carbonyl reductase (EC.1.1.1.184.), respectively (Wermuth, 1981; Vander Jagt et al., 1990a, 1992). All have a broad substrate specificity, are located in cytosol and share the feature of $NADP^+$ requirement (Wermuth, 1981; Vander Jagt et al., 1990a, 1992). Interestingly, eventhough the reduction of methylglyoxal by aldose reductase produces 95% acetol, D-lactaldehyde is also formed in an amount of as many as 5% (Vander Jagt et al., 1992). This fact is particularly noteworthy as usually L-lactaldehyde is generated.

The family of aldehyde dehydrogenases (EC.1.2.1.3.), consists of three isoenzymes, designated E1, E2 and E3, differing in their intracellular locations (Izaguirre et al., 1998). Methylglyoxal has been reported to be a substrate for all of them (Izaguirre et al., 1998). Beside the above mentioned groups of enzymes there are lot of other dehydrogenases and reductases which have been shown to catalyze the reduction of pyruvaldehyde. Sometimes it is not clear how these enzymes, e.g. sepiapterin reductase (Sueoka and Katoh, 1995), methylglyoxal reductase (Bender et al., 1994), etc., are related to the above discussed ones whereby the nomenclature may be a bit confusing and certainly further endeavours are needed to clear up the situation.

As a specific oxido-reductase, a single enzyme catalyzing the transformation of methylglyoxal into D-lactate has been reported from *E. coli* (Misra et al., 1995). The enzyme, designated glyoxalase III, is active without glutathione (Misra et al., 1995). Although, such an activity in different *E. coli* strains has been reported from another laboratory too (MacLean et al., 1998), a further confirmation of its existence is needed.

Recently, glyoxal oxidase has been described in *Phanerochaete chryssosporium* as a new H_2O_2 producing enzyme that uses up aldehydes as substrates, among others methylglyoxal too (Kersten and Kirk, 1987).

Finally, it is to be noted that pyruvate dehydrogenase complex also has the ability of methylglyoxal oxidation (Baggetto and Lehninger, 1987), but in contrast to an earlier report (Uotilla and Koivusalo, 1974), methylglyoxal does not function as a substrate for formaldehyde dehydrogenase (Pourmotabbed and Creighton, 1986).

3.3. Non-enzymatic methylglyoxal production

3.3.1. Uncatalyzed dismutation of trioses and triose-phosphates into methylglyoxal

The non-enzymatic formation of methylglyoxal from glyceraldehyde and dihydroxyacetone was first observed around the turn of our century (Patterson and Lazarow, 1955; Speck, 1958). Later, the feasibility of these dehydrations was confirmed in different buffers at various pHs (Needham and Lehman, 1937; Speck, 1958; Riddle and Lorenz, 1968). However, in the above mentioned experiments 2,4-dinitrophenylhydrazine was used for the determination of methylglyoxal (Riddle and Lorenz, 1968) and this was the reason of why doubts emerged concerning the possibility of reactions (Thornalley, 1985). It was suggested, mostly based on the observations made during the investigation of autoxidation of simple

monosaccharides such as glyceraldehyde, dihydroxyacetone and glycolaldehyde, that methylglyoxal cannot be formed from glyceraldehyde and dihydroxyacetone in the reported system under physiological pH but it is rather an experimental artefact due to the dehydration of trioses by acidified 2,4-dinitrophenylhydrazine (Thornalley, 1985). In addition, letting trioses to be autoxidized hydroxymethylglyoxal was asserted to be the product of reaction, a compound being on the one hand a substrate for glyoxalase I and on the other hand capable of interacting with 2,4-dinitrophenylhydrazine (Thornalley, 1985). It is noteworthy, Bonsignore et al. (1970) used glyoxalase I to determine the amount of methylglyoxal generated in the experiments with glyceraldehyde exposed to lysine as catalyst. They definitely demonstrated the formation of a product being metabolized by glyoxalase I (Bonsignore et al., 1970). Nevertheless, the suggestion that the formation of methylglyoxal from trioses is artificial, gained a support from the findings of Ohmori et al. (1989). They repeated the experiments of Riddle and Lorenz (1968). It was found that methylglyoxal was detectable only in that case when 2,4-dinitrophenylhydrazine was used for the assay of α-oxoaldehyde (Ohmori et al., 1989). When gas chromatography was applied to detect methylglyoxal, they failed to show any formation of methylglyoxal (Ohmori et al., 1989). To make the situation more conflicting, the dehydrations of trioses to methylglyoxal were proven in a series of experiments by using o-phenylendiamine for derivatization of the product (Fedoronko and Königstein, 1969, 1971). A general acid-base catalysis was supposed for the reaction (Fedoronko and Königstein, 1969, 1971). Otherwise remarkable is the fact that o-phenylendiamine is a compound which is used for methylglyoxal derivatization in an advanced HPLC technique adviced for the detection of methylglyoxal in biological systems (Ohmori et al., 1987a). To sum up, the feasibility of reaction under physiological circumstances is a questionmark, while under pathological/special conditions it seems to be possible.

In the Embden–Meyerhof scheme of glucose breakdown a subordinate role was ascribed to methylglyoxal (Meyerhof, 1948). Hence, it is not a surprise that the note which pointed out the probability of a non-enzymatic generation of methylglyoxal from glyceraldehyde 3-phosphate, remained unnoticed (Meyerhof and Lohman, 1934). After more than 30 years of apparent death of interest the possible significance of this reaction has been recognized and some of its characteristics have been described (Bonsignore et al., 1973). However, only by the 1990s became it clear how this reaction proceeds. Now, it is accepted that the deprotonation of substrate to an enediolate phosphate followed by the cleavage of phosphate group from the carbon skeleton result in the formation of an enol form of methylglyoxal (Richard, 1993). An estimate for the rate of non-enzymatic formation of methylglyoxal is given as many as 0.1 mM/day (Richard, 1993).

3.3.2. Methylglyoxal generation from acetoacetate in reactions leading to free radical formation

It was Milligan and Baldwin (1967), who discovered the possible non-enzymatic conversion of acetoacetate into methylglyoxal in the presence of molecular oxygen and myoglobin. Free radical generation was suggested to be involved in the reaction mechanism and specific requirement for manganase was found too (Milligan and Baldwin, 1967). Yet hemoglobin and cytochrome c also gave rise to the transformation of acetoacetate to methylglyoxal, but their effectivity as catalyst was less than that of myoglobin (Milligan and Baldwin, 1967). Based on these findings the traits of catalysis were set in an analogy to the characteristics of α-oxidase system in plants (Milligan and Baldwin, 1967). As a whole, the reaction was thought to have a feasible role in decarboxylation of acetoacetate, but the in vivo significance of it was questioned (Milligan and Baldwin, 1967). Later other examinations revealed some further features of this decarboxylation (Vidigal and Cilento, 1975; Takayama et al., 1976). Namely, dioxetane ring formation, a strict correlation among light emission, methylglyoxal generation and O_2 uptake were described, and the destruction of myoglobin was noted to be due to a photochemistry without light reaction being a consequence of the generation of excited methylglyoxal during catalysis (Vidigal and Cilento,

1975; Takayama et al., 1976). In addition, myoglobin was possible to be replaced with peroxidase and catalase, but light emission was only observed in case of peroxidase (Takayama et al., 1976). And further, acetoacetate was described as an electron donor molecule in the peroxidase system (Harrison and Saeed, 1981). Indeed, the biological significance of reactions mentioned here is not known, but a feasibile implication may be their involvement in the development of diabetic complications.

3.4. Methylglyoxal production in biotransformation of xenobiotics

Thiazolone derivatives are known as useful antihelmintic and fungicide compounds in agriculture, in animal husbandry and as ingredients in various consumer products (Mizutani et al., 1993). These compounds may undergo microsomal epoxidation followed by hydrolysis and ring cleavage, thus leading to the formation of thioamides and α-oxoaldehydes, like methylglyoxal (Mizutani et al., 1993). Eventhough only a low percent of the administered 2-(*p*-methoxyphenyl)-4-methylthiazole were quantitatively recovered as methylglyoxal in the collected urine of mice, it should be kept in mind that most thiazoles are expected to enter the cells by passive diffusion and transformed into toxic metabolites inside the cells, particularly in hepatocytes (Mizutani et al., 1993). Since the glyoxalase system is very effective, especially in the liver, the low urinary excretion rate of methylglyoxal in the experiments does not necessarily instruct us upon either the rate of methylglyoxal liberation or the hepatotoxicity might have caused by thiazolones. The toxicological significance of these findings is to direct attention to minor but under specific circumstances important events as biotransformation of xenobiotics.

4. Toxicology of methylglyoxal

4.1. Methylglyoxal production as an environmental hazard

From toxicological point of view it is needed to take into consideration those possible ways of methylglyoxal production which in essence happen outside the living organisms, but the consequence of those may be an in vivo methylglyoxal burden.

The improvement of analytical methods has made possible the investigation of the existence of carbonyl products in wet deposition and water, and also in urban atmosphere. Methylglyoxal has been identified in rainwater, fogwater and mist samples from South-California in Los Angeles area (Steinberg and Kaplan, 1984; Igawa et al., 1989). Indeed, it added substantial contribution only to the contamination of fog and mist, while formaldehyde and glyoxal were the predominant aldehydes detected in rainwater (Steinberg and Kaplan, 1984; Igawa et al., 1989). The urban atmosphere has to be considered as a source of aldehydes, since the concentrations of carbonyls were greater at the inland site of investigated region than along the coast (Igawa et al., 1989). Probably, the rainwater droplets took up carbonyls from the polluted air and conveyed them to the groud as wet precipitate. Some of the aldehydes (e.g. formaldehyde, acetaldehyde) are directly formed in the internal combustion engines, but methylglyoxal is not (Steinberg and Kaplan, 1984). It is rather one of the products from the reaction of ozone with aromatic hydrocarbons and toluene, compounds generally found in motor exhaust due to the incomplete combustion (Atkinson et al., 1980; Steinberg and Kaplan, 1984). And also, the decomposition of aromatic compounds, like toluene as modelled in smog chamber experiments, leads to methylglyoxal production, which is particularly sensitive to photooxidation resulting in the propagation of radical generation (Carter et al., 1979; Atkinson et al., 1980). Thus, methylglyoxal has more impact in toxic events than could be assigned to by only considering its concentration.

Another point is the ozonation and chlorination of watery solutions of humic substances resulting also in the production of a wide variety of aldehydes and ketones, among which methylglyoxal also takes place (Matsuda et al., 1992a,b). If the fact is taken into consideration that in water plants the ozonation and/or chlorination are usu-

ally applied to the removal of tastes, odors and color (Matsuda et al., 1992a,b), then it is clear why it is compelling to look at methylglyoxal as a potential environmental hazard eventhough according to a recent report of IARC methylglyoxal has not been detected in drinking-water (IARC Working Group on the evaluation of carcinogenic risks to humans, 1991).

Last but not least, a wide variety of foodstuffs, consumers' goods and beverages contain methylglyoxal [for review see IARC Working Group on the evaluation of carcinogenic risks to humans (1991)] and it is present in cigarette smoke, too (Moree-Testa and Saint-Jalm, 1981).

4.2. Methylglyoxal toxicity in vivo

4.2.1. In mammals

The first experiments upon the in vivo toxicity of methylglyoxal led to the conclusion that the subcutaneous injection of this aldehyde had no effects on rabbits (Dakin and Dudley, 1913b). However, other studies revealed the toxicity of methylglyoxal. Lethal doses of this α-oxoaldehyde resulted in characteristic dissectional findings, namely, pulmonal hyperemia and edema were generally found with concomittant but not obligatory appearance of liver, kidney and intestine degenerations (Sjolemma and Seekles, 1926). In mice, when single doses of methylglyoxal were given intraperitoneally, 800 mg/kg body weight was lethal to the animals within 4 h, while in animals treated with less than the lethal dose, after 24 h there was a decrease of liver weight proportional to the dose administered (Kalapos et al., 1991a; Choudhary et al., 1997). In addition, pulmonal hyperemia with distended, fibrin-covered small intestines were found in the aldehyde injected mice, which otherwise was less explicit when 48-h fasted mice were injected with methylglyoxal 24 h prior to sacrifice (Kalapos, 1994a). Nevertheless, not any purulent fluid or any other sign of inflammation could be ascertained. In the paired-control groups receiving physiological saline not any fibrin-cover, adhesion or distension of intestinal tract were seen (unpublished). Twenty-four hours after the injection of 400 mg/kg body weight dose of methylglyoxal the light microscopic examinations showed slight histological changes in murine livers dominated by vacuole formation in the centrilobular areas [Kalapos et al. (1991a) and unpublished observations]. A semiquantitative scaling of morphological changes in periodo-acid Schiff reaction (PAS) stained sections revealed that methylglyoxal pretreatment of mice led to a decrease of staining intensity suggesting a glycogen loss (unpublished). When Epon 812 embedded sections were analyzed for the characterization of vacuoles, it turned out that vacuole formation mostly appeared at the vascular region of hepatocytes (unpublished). Oral administration of 0.2% (w/v) pyruvaldehyde in drinking water for 6 weeks resulted in similar effect on liver weight of rats only in that case when it was added after a diethylnitrosamine (DEN) injection (Hasegawa et al., 1995).

Oral administration of methylglyoxal to female mice led to collagen accumulation in kidneys resulting in glomerular basement membrane thickening (Golej et al., 1998), an obligatory morphological change of kidneys in diabetics (Osterby, 1986). The fluorescence of extracted proteins was about twice as high as in controls (Golej et al., 1998), a characteristics of collagen originating from diabetic animals (Brennan, 1989). Furthermore, numerous studies indicate the in vivo generation of fluorescent products in humans, the formation of which is a result of the interaction between methylglyoxal and protein side chains (Nagaraj et al., 1996; Ahmed et al., 1997; Uchida et al., 1997; Shamsi et al., 1998). Methylglyoxal forms imidazolysine and carboxyethyllysine, and argpyrimidine, 5-hydro-5-methylimidazolone and 5-methyl-imidazolone with lysine and arginine residues of proteins, respectively, by Maillard reaction (Nagaraj et al., 1996; Ahmed et al., 1997; Uchida et al., 1997; Shamsi et al., 1998). Antibodies raised against some of these epitopes have enabled to identify dicarbonyl modified proteins both in arterial as well as corneal tissues and in plasma (Uchida et al., 1997; Shamsi et al., 1998). In support to this scheme, a positive correlation between methylgly-

oxal derived modifications of plasma proteins and glycohemoglobin concentration has also been demonstrated underlying the belief that the in vivo formation of this dicarbonyl is dependent on the measure of glycemia and likely contributes to complications evolving in diabetes mellitus (Uchida et al., 1997; Shamsi et al., 1998).

The psychomotoric activity of methylglyoxal treated animals changed. As described for rabbits, the animals were without appetite and depressed (Sjolemma and Seekles, 1926). In mice, the movement was slowed down and ataxy was observed (Kalapos et al., 1991a).

The influence of in vivo added methyglyoxal on enzymatic activities was also investigated. Intraperitoneal administration of methylglyoxal proved to influence the hepatic microsomal monooxygenase system in mouse (Bronzetti et al., 1987; Kalapos et al., 1991a). Nevertheless, there is no agreement on how the isozymes of cytochrome P-450 superfamily are modified as in one study all the tested activities, including aminopyrine *N*-demethylase and aniline hydroxylase, were elevated in female mice, while in an other study in which male rodents were used, an increase of aniline hydroxylase activity was documented with a concomitant fall of aminopyrine *N*-demethylase activity (Bronzetti et al., 1987; Kalapos et al., 1991a). As discussed in an other paper, this discrepancy may have been due to the different sex of mice used (Kalapos, 1994a). This suggestion receives a support from the fact that the increase of aniline hydroxylase activity in the liver has been observed in rodents with chemically-induced diabetes mellitus (Kato et al., 1970; Reinke et al., 1978), while the hepatic microsomal aminopyrine *N*-demethylase activity was depressed in streptozotocin-induced diabetic male rats, but not in female ones as in those it was increased over the contols (Reinke et al., 1978). Methylglyoxal diminished in a time- and dose-dependent manner the specific activities of superoxide dismutase, glutathione S-transferase (GST), catalase, glyoxalase I and II in murine livers (Choudhary et al., 1997). In spleen, a deteroriation of catalase and glyoxalase I activities was only seen (Choudhary et al., 1997). The suppression of above discussed enzymatic activities by methylglyoxal was reproduced in an in vitro system, too (Choudhary et al., 1997). Interestingly, the effect of α-oxoaldehyde on DT-diaphorase activity was somewhat different from the mode of action seen above. Namely, in the liver, low doses of the aldehyde increased the enzymatic activity, while doses higher than 100 mg/kg body weight deteriorated that (Choudhary et al., 1997). In spleen, the fall of DT-diaphorase activity missed (Choudhary et al., 1997).

The administration of increasing concentrations of methylglyoxal depressed the amount of GSH in murine livers within 6 h, while this effect of the aldehyde appeared only after 12 h in spleens (Kalapos et al., 1991a; Choudhary et al., 1997). Though, similarly to in vitro observations (Kalapos et al., 1992a), GSH concentration showed a recovery to the normal level in the liver, the phenomenon was not seen in the spleen (Choudhary et al., 1997); maybe due to the fact that the experiments were finished before the recovery of GSH could have started in this organ. Noteworthy is the fact that even the highest rate of the depletion of tripeptide stores did not decrease below 50% of the original GSH level in either of the cases, which may be an explanation of why only a moderate elevation of serum glutamate pyruvate transaminase activity was detected (Kalapos et al., 1991a; Choudhary et al., 1997). Parallel to the changes of GSH levels, significant increase in the amount of lipid peroxidation product malondialdehyde was found both in the livers and, delayed in time, in the spleens of animals (Choudhary et al., 1997). Here it is appropriate to notice that in an earlier paper a bad fluctuation of the measured amounts of malondialdehyde was reported when lipid peroxidation was followed in isolated murine hepatocytes after their treatment with increasing concentrations of methylglyoxal (Braun et al., 1994). Also to be noted in relation to diabetes, both an increase of malondialdehyde levels and a fall of GSH concentrations have been reported in diabetic animals (Oberley, 1988). Despite being attentive to the doubts and uncertainties, it is attractive to think that methylglyoxal exerts some in vivo effects resembling and mimicking the events of an oxidative pressure, which generally happens in diabetic state.

The effects of methylglyoxal on carcinogenesis have been investigated in three animal models: in skin and gastric tumorigenesis models, and in liver bioassay model (Furihata et al., 1985; Nagao et al., 1986; Martelli et al., 1988; Takahashi et al., 1989; Miyakawa et al., 1991; Hasegawa et al., 1995). Beside the histological examinations, induced ornithine decarboxylase (OCD) activity and stimulated DNA synthesis are shown as useful markers of possible tumour promoting activity of chemicals (Furihata et al., 1985; Takahashi et al., 1989). γ-Glutamyl transpeptidase (γ-GT) and glutathione S-transferase placental form (GST-P) are also good markers for the detection of putative preneoplastic lesions (Martelli et al., 1988; Hasegawa et al., 1995).

In a long-term two-stage skin model in which femal mice were subcutaneously injected with a total dose of 500 μmol methylglyoxal for 5 weeks and subsequently treated with 12-O-tetradecanoylphorbol-13-acetate for 47 weeks, the dicarbonyl did not initiate tumour generation in the skin of animals (Miyakawa et al., 1991). This fact was consistent with the calculated ID_{50} value, which proved the highest for methylglyoxal among the series of tested carcinogenic agents (Miyakawa et al., 1991). However, the clastogenic ability (CD_{20}) of methylglyoxal was surprisingly high in comparison to other test compounds (Miyakawa et al., 1991). CD_{20} value was estimated by linear extrapolation from the percentages of chromosome aberrations using Chinese hamster V79 cells (Miyakawa et al., 1991). The reason for poor correlation between ID_{50} and CD_{20} values was not clear for the authors (Miyakawa et al., 1991). In a simple skin model experiment, 2 mg methylglyoxal was injected into the skin of 18 rats twice a week for 10 weeks (Nagao et al., 1986). After 20 months, 3 males and 1 female out of the methylglyoxal treated group of animals developed cancers at the site of injection, while the saline injected controls were without tumours (Nagao et al., 1986). Histologically the tumours proved to be fibrosarcoma (Nagao et al., 1986).

The effect of per os administered methylglyoxal on gastric tumorigenesis in male Wistar rats was also examined. After an 8-week period of initiation with *N*-metil-*N′*-nitro-*N*-nitrosoguanidine (MNNG) 0.25% methylglyoxal was added to the animals in their drinking water for 32 weeks and the dicarbonyl was without any apparent enhancing effect on the yield of adenocarcinomas as compared to the respective controls (Takahashi et al., 1989). However, the incidence of hyperplasia in the pyloric region was significantly raised, while in the fundus it was ineffective in this regard too (Takahashi et al., 1989). Without MNNG initiation methylglyoxal did not cause any histological change in pyloric mucosa either (Takahashi et al., 1989). The oral administration of methylglyoxal by gastric tube at single doses ranging 300–600 mg/kg body weight led to a dose-dependent rise of unscheduled DNA synthesis within 16 h and induced OCD activity by 100-fold within 7 h in rat glandular stomach mucosa (Furihata et al., 1985). After methylglyoxal administration had been finished DNA synthesis returned to the original rate within 24 h (Furihata et al., 1985). The morphological analysis of tissues specimens revealed an atrophy of pyloric mucosa with a concomitant cell necrosis (Furihata et al., 1985). It was concluded that these results set out a potential promoter activity for methylglyoxal in carcinogenesis, eventhough the aldehyde was less effective with respect to other well known carcinogenic agents (Furihata et al., 1985).

Methylglyoxal (0.2%) added in drinking water increased the numbers of GST-P positive foci only in that case when the animals were injected with DEN prior to the study in a mid-term liver bioassay experiment with male rats being subjected to a 2/3 hepatectomy at week 3 (Hasegawa et al., 1995). In an other study, rats were on a diet of 2-acetylaminofluorene (2-AAF) for 2 weeks with CCl_4 administration at the mid-point and 1 week after the end of 2-AAF feeding different concentrations of methylglyoxal were added to the animals in tap water (Martelli et al., 1988). On day 28, the rats were partially hepatectomized (Martelli et al., 1988). Methylglyoxal caused a concentration dependent increase in the number of γ-GT positive foci in the livers in comparison to animals either without initiation with 2-AAF or without any pretreatment with chemicals (Martelli et al., 1988). Methylglyoxal applied in

combination of hepatectomy either as an initiator or as a promoter raised the number of foci (Martelli et al., 1988). On this basis the authors arrived at the conclusion that methylglyoxal may have been a complete carcinogen in rat liver (Martelli et al., 1988).

The presented results are variable and the conclusions drawn prompt quite different behaviour of methylglyoxal in various systems. Although these inconsistent data may be at least in part due to the different experimental conditions and also to the relatively low number of animals involved in the studies, the variability is probably also owing to the mode of action of the dicarbonyl (see Section 5.2).

Methylglyoxal exerts an anti-tumour activity in vivo. When it was given intraperitoneally or intravenously the arrest of tumour growth and the reduction of tumour size were observed in animals bearing different types of malignant cells in their abdominal cavities (e.g. Ehrlich ascites tumour cells) or bearing subcutaneously implated mamma carcinomas (Apple and Greenberg, 1967; Jerzykowski et al., 1970; Conroy, 1978; Dianzani, 1978). These findings urged scientists to work out strategies in order to increase methylglyoxal concentration in cancerous cells. Since large body of data have been gathered in this field the inquiring readers are advised to consult the numerous papers available on this topic (Ly et al., 1998). Nevertheless, it has to be stressed that to date the problem of selective toxicity against tumour cells, which is a necessary condition to any clinical application, has not been solved. Until this criterium is not fulfilled these compounds can only be regarded as useful tools in biochemical research, but not as possible medicines in clinical practice.

In relation to cancer it still has to be added that the activities of both glyoxalase I and II decreased in the livers of mice being implanted intraperitoneally with different types of tumour cells and the alteration of enzymatic activities was accompanied by an elevation of intrahepatic methylglyoxal concentration (Strzinek et al., 1970; Winter et al., 1978; Matsuura et al., 1986). These phenomena became apparent only after few days the implantation had been perfected (Strzinek et al., 1970; Winter et al., 1978; Matsuura et al., 1986). It is also remarkable that the glyoxalase activities of red blood cells of tumour bearers were changed in a quite hectic manner (Piskorska and Kochanski, 1989). Yet the significance of these observations is not clear.

4.2.2. In yeast

Extensive bioengineering work has been done with yeast, and the enzymes involved in methylglyoxal metabolism and discussed under Section 3.1 are present in different yeast strains with the only exception of acetone metabolizing cytochromes (for review see Inoue and Kimura, 1995; Inoue et al., 1992). The best characterized strain is *S. cerevisiae* (Inoue and Kimura, 1995). In most of the cases the behaviour of enzymes does not differ significantly from the general view presented, but some specificities for glyoxalase I have to be listed. Firstly, a gene, named glyoxalase I activation conferring gene, has been indentified and the function of its transcript is believed to be related somehow to the increase of glyoxalase I activity by influencing mRNA level of the enzyme, thus affecting cell size and resistance to methylglyoxal (Murata et al., 1988). Secondly, a mating factor induced phosphorylation of glyoxalase I has been communicated (Inoue et al., 1990) and thirdly, cell–cell interactions have an effect on glyoxalase I activity, higher is cell density glyoxalase I increases accordingly (Inoue and Kimura, 1995). Growth arrest and cell killing of a glyoxalase I deficient mutant strain of *S. cerivisiae* were noticed when the cells were exposed to glycerol or methylglyoxal, while in the parent strain cultured in either glycerol or methylglyoxal containing media a several-fold induction of glyoxalase I was communicated (Penninckx et al., 1983). These findings give an indication for the essential role of glyoxalase system in dicarbonyl detoxification in yeast (Penninckx et al., 1983). The growth of *Hansenula mrakii* was irreversibly inhibited by methylglyoxal only when 50 mM aldehyde was added to the culture medium (Inoue et al., 1991).

4.2.3. In plants

Although plant cells have been thoroughly examined in culture for the development of methylglyoxal toxicity [for review see Deswal et al. (1993)], whole plants themselves have barely been investigated in this regard. What at the present status of research is known in this field, is too little. Methylglyoxal had an inhibitory effect on germination of barley and the inhibition was proportional to the concentration of aldehyde (Mankikar and Rangekar, 1974). At low concentration of inhibitor the signs of recovery could be demonstrated, whereas the inhibition became complete and irreversible at concentrations higher than 1 μM (Mankikar and Rangekar, 1974). Evidently, the addition of cysteine, just like in experiments with protozoa and bacteria (see later), impeded the development of the inhibitory action of dicarbonyl (Mankikar and Rangekar, 1974). In the case of cultivation of pine tumours, methylglyoxal in combination with ascorbic acid promoted the development of bud primordia from the tumours while inhibited the growth of neoplastic tissues (Lieber, 1995). With other plant tumours the synergistic action of the above two compounds on bud and plantlet developments was repeatedly demonstrated (Lieber, 1995). Unfortunately, further details from the above experiments are not available at this time. Recently, in hydroponically grown tomato subjected to different stressors like salt stress or water deficit an increase of the expression of glyoxalase I was found (Espartero et al., 1995). A quite equal distribution of enzyme was revealed by immunhistology regardless of whether proliferating or non-proliferating tissues were analyzed (Espartero et al., 1995). The authors concluded that the reduction of plant growth induced the expression of glyoxalase I which was otherwise only a mirror of a higher demand for active metabolism (Espartero et al., 1995). Taking together data upon the induction of glyoxalase I in various biological systems a generalization of the idea has been made, namely, in all the cases when a bad demand for energy should be satisfied, glyoxalase I is going to be induced thus protecting cells against methylglyoxal ineradicably produced as a by-product of highly operative glycolysis (Kalapos, 1999a).

4.2.4. In parasites

So far the investigation of methylglyoxal metabolism in parasites has not been in the centre of interest. To date, we are unaware of the existence of methylglyoxal synthase in protozoa, whereas the enzymes involved in the degradation of this dicarbonyl are present in a substantial amount (Ghoshal et al., 1989; Vander Jagt et al., 1990b). When *Leishmania donovani* in which glycolytic path was inhibited, was incubated with glucose, glycerol or dihydroxyacetone phosphate as sources of energy neither methylglyoxal nor D-lactate accumulation could be detected (Ghoshal et al., 1989). In another study, protozoa, particularly *Leishmania* species and *Trypanosoma lewisi* grown on glycerol, released D-lactate into the incubation medium both in aerobiosis and in anaerobiosis showing the existence of an active glyoxalase system in these species (Darling et al., 1988; Cazzulo, 1992). This fact is further substantiated by the efficient catabolism of externally added methylglyoxal (Ghoshal et al., 1989). Nevertheless, methylglyoxal exerts toxic effects to protozoa by inhibiting their growth or diminishing their motility and rounding their shape (Sarkar and Bhaduri, 1986; Darling and Blum, 1988; Ghoshal et al., 1989). Indeed, its action on motility should be taken into account very carefully as manufacturer's product was used without any purification (Darling and Blum, 1988). The growth inhibitory effect of dicarbonyl was concentration dependent and reversed by the addition of cysteine to the medium (Sarkar and Bhaduri, 1986). In summary, it seems that glyoxalases are probably very crucial in the adaptation of protozoa to environmental circumstances and D-lactate production functions as a defense mechanism.

The situation is very confusing with respect to the distribution of glyoxalases in helmints as neither in *Cestodes* nor in *Digeneans* active glyoxalase I can be detected while glyoxalase II is obviously present (Brophy et al., 1990). In *Nematodes*, however, both enzymes are detactable (Brophy et al., 1990). To our best knowledge, toxicological studies have not been undertaken with helmints.

4.2.5. In procaryotes

Procaryotes harbor the majority of enzymes listed in Section 3.1. The metabolism of acetone is also possible whereas it is a questionmark whether acetone monooxygenase or perhaps other enzymes are the catalysts converting acetone into acetol (Taylor et al., 1980). Acetol is metabolized by acetol dehydrogenase using NAD^+ as cofactor (Taylor et al., 1980).

Cell growth inhibitory and cell killing effects of methylglyoxal to procaryotic cells have been known for quite a long time (Bloch et al., 1945; Együd and Szent-Györgyi, 1966a). The effect of methylglyoxal on the proliferation of *E.coli* was shown to be dose-dependent and reversed by the addition of cysteine (Együd and Szent-Györgyi, 1966a). The concentration of methylglyoxal which already exerted a marked inhibiting effect on cell division, did not affect the oxidative metabolism (Együd and Szent-Györgyi, 1966a). Thus, it was concluded that the observed inhibition of growth was due to the inhibition of cell division machinery itself and not to the action of aldehyde on metabolism (Együd and Szent-Györgyi, 1966a). As noted, however, DNA replication and protein synthesis were inhibited by methylglyoxal in *E. coli* and *P(revotela) ruminicola* (Russell and Cook, 1995; Együd and Szent-Györgyi, 1966b), and also a decline was caused by the aldehyde in intracellular potassium concentration ($[K^+]_{IC}$) and in membrane potential (Russell, 1993). Observation was also made that methylglyoxal sensitized bacterial cells to ionizing radiation (Ashwood-Smith et al., 1967).

Now that some of the effects of methylglyoxal have been established, we are in the position to begin discussing the mechanisms of methylglyoxal toxicity. However, we limit our discussion to the studies on glycerol and carbohydrate metabolism as well as on regulation of intracellular potassium concentration.

In an *E. coli* strain having lost the ability to control the rate of synthesis of glycerol kinase and the feedback inhibition of the enzyme by fructose 1,6-bisphosphate produced an overwhelming amount of methylglyoxal when cells were grown on glycerol (Freedberg et al., 1971; Krymkiewicz et al., 1971). Methylglyoxal formation was also shown in the case of various carbon sources too (Freedberg et al., 1971). When methylglyoxal resistant mutants were selected out, unlike their parents all the resistant strains had an increased glutathione dependent activity to remove the aldehyde and became immune to glycerol (Freedberg et al., 1971). The existence of *Salmonella typhi* strains being hardened to methylglyoxal was also reported (Hooke et al., 1993). High level of glycerol kinase expression was also documented in a strain of *Bacillus stearothermophilus* being badly sensitive to glycerol (Burke and Tempest, 1990). A severe deterioration of both respiration and cell growth was found when cells were exposed to glycerol (Burke and Tempest, 1990). Interestingly, the P_i limited cultures compared to glycerol limited cultures were less sensitive to the fluctuations of environment and the glycerol kinase activity was lower in these cases, but the methylglyoxal synthase and glyoxalase activities were raised (Burke and Tempest, 1990). In an *E. coli* strain constructed by the introduction of a plasmid holding methylglyoxal synthase gene (mgsA) cloned under trc promoter the activity of synthase was 25-fold and more than 400-fold higher in the absence and in the presence of isopropylthiogalactoside (IPTG), respectively, than in the parent cells (Tötemeyer et al., 1998). The growth of transformed cells was uneffected when cultured in liquid medium with glycerol as sole carbon source, but the supplementation of medium with IPTG led to the accumulation of methylglyoxal and cessation of cell growth (Tötemeyer et al., 1998). A possible couple of intracellular P_i pools to toxic events was raised suggesting that the high activity of glycerol kinase through the generation of high levels of dihydroxyacetone phosphate results in an increase of $NADH + H^+$ and consequently oxygen consumption also rises resulting in ATP synthesis and depleting P_i pools (Freedberg et al., 1971; Burke and Tempest, 1990). The fall of P_i concentration activates methylglyoxal synthase and on the one hand, generates methylglyoxal while on the other hand, liberates P_i for the kinase (Freedberg et al., 1971; Burke and Tempest, 1990). If the methylglyoxal detoxifying capacity is insufficient the cells are exposed to the deteriorating action of a hazardous compound, as described above.

The toxicity of glucose to *P. ruminicola* cells was observed when cultured in N-limited medium having an excess of glucose (Russell, 1992). The parent strain (B1-M) grown in the presence of increasing concentrations of glucose started accumulating methylglyoxal and its growth slowed down when glucose concentration reached 10 mM (Russell, 1993). The accumulation of methylglyoxal was in a good correlation with the loss of cell viability and the increasing concentration of P_i was without any effect on that (Russell, 1993). The mutant strain (B1-R) harboring threefold less β-glucosidase than the parent one accumulated about 1/3 of methylglyoxal found in the case of previously mentioned strain (Russell, 1993). The externally added methylglyoxal had the same effects as seen in case of glucose toxicity, namely ATP concentration first increased and then dropped, while the glucose transport activity, the membrane potential as well as the $[K^+]_{IC}$ fell (Russell, 1993). Both the wild type and the mutant were equally sensitive to the externally added aldehyde (Russell, 1993). The overexpression of methylglyoxal synthase in *E. coli* did not lead to any obvious effect of glucose on cell growing, while xylose substantially depressed cell division and decreased cell viability (Tötemeyer et al., 1998). When the uhpT gene that encodes an active transport for sugar phosphates in *E. coli* and is a subject of catabolite repression, was carried in a multicopy plasmid the growth of strains was impeded upon addition of various sugar phosphates such as D-glucose-6-phosphate, D-fructose-6-phosphate, D-mannose-6-phosphate (Kadner et al., 1992). Cell killing was also detected and the lethality occured as a response to amplification of sugar derivative transport and correlated with methylglyoxal production (Kadner et al., 1992). Though D-ribose-5-phosphate, D-galactosamine-6-phosphate and other sugar-phosphates resulted in an inhibition of cell growth but neither the loss of viability nor the accumulation of dicarbonyl were seen (Kadner et al., 1992). In *Klebsiella pneumoniae* cells cultured in 2-ketogluconate limited medium under anaerobic conditions the simultaneous functioning of pentose-phosphate and Entner–Duodoroff pathways was suggested as catabolic routes (Simons et al., 1992). When these cells were exposed to a pulse of 2-ketogluconate the relief of carbon source limited conditions led to lactate production which partly happened via the glyoxalase path (Simons et al., 1992). Summarizing the findings on the energetics of bacterial growth a mechanism to give an explanation of why methylglyoxal is generated in bacteria was presented. According to this hypothesis methylglyoxal production is a result of the imbalance between anabolic and catabolic processes (Russell, 1993). Namely, if there is an inadequate amount of ADP to operate the ATP-generating part of glycolysis (excess of ATP) then triosephosphates are converted into methylglyoxal and the energy spilling via glyoxalases protects bacterial cells from the toxic electrophile (Russell, 1993).

In relation to methylglyoxal toxicity the effect of cAMP on sugar metabolism was examined thouroughly. A wild type strain of *E. coli* K-12 accumulated a toxic level of methylglyoxal when cultured in medium containing one of D-xylose, L-arabinose and D-glucose-6-phosphate in the presence of cAMP (Ackerman et al., 1974). In the case of D-xylose methylglyoxal accumulation was associated with an elevated rate of sugar uptake (Ackerman et al., 1974). A strain carrying a deletion in the gene encoding adenylate cyclase (cya) and a mutation in cAMP receptor protein (crp) selected for growth on L-arabinose accumulated 2.5 times as much as methylglyoxal as the wild type when cultured in a D-glucose-6-phosphate minimal medium (Puskas et al., 1983). The toxicity of methylglyoxal could not be attributed to the changes in activities of methylglyoxal producing and degrading enzymes (Puskas et al., 1983). However, an elevated phosphofructokinase activity was found leading to an elevated dihydroxyacetone-phosphate level in the mutant strain which may explain the increased rate of methylglyoxal generation (Puskas et al., 1983). It is remarkable that the addition of cAMP to the medium resulted in higher levels of both methylglyoxal synthase and glyoxalases in both strains (Puskas et al., 1983). Using cAMP suppressor mutants, spontaneous pseudorevertants of adenylate cyclase negative strains, a functionally active cAMP–cAMP receptor protein complex was

needed for the manifestation of methylglyoxal accumulation in the cells grown on minimal L-arabinose medium (George and Melton, 1986). In the lack of cAMP the growth of *E. coli* strains of ($kefB^+$, $kefC^+$) and ($kefB^-$, $kefC^-$) cultivated on xylose was indistinguishable (Ferguson et al., 1993). The addition of cyclic nucleotide provoked a marked decrease of growth in both strains but the loss of viability in Kef positive strain evolved later than in the mutant lacking active Kef system (Ferguson et al., 1993). In a null strain for glyoxalase I (ΔgloA) the addition of cAMP and L-xylose elicited an immediate and irreversible loss of cell growth and viability (MacLean et al., 1998). The 20-fold overexpression of methylglyoxal synthase led to a slight impairement of bacterial growth on L-xylose and glycerol, while the coupling of overexpression with the addition of cAMP cessation of growth was evidently present on L-arabinose, glycerol and L-xylose and in a moderate degree on D-ribose (Tötemeyer et al., 1998). The growth of a null mutant for methylglyoxal synthase (mgsA::kan) was inhibited by cAMP when cells were cultivated on L-xylose, whereas there were neither loss of cell viability nor synthesis of methylglyoxal (Tötemeyer et al., 1998). Without cAMP the null mutant cells grew on L-xylose over a 300 min period of time (Tötemeyer et al., 1998).

Over the last decade Booth et al. investigated the interrelationship between methylglyoxal toxicity and potassium household in *E. coli*. Methylglyoxal posed a transient loss of intracellular potassium, but did not have an effect on the reversal of normal potassium level (Ferguson et al., 1993). An active KefB was a prerequisite for methylglyoxal initiated loss of potassium and was protective against the electrophile (Ferguson et al., 1993). The addition of methylglyoxal to the culture medium activated KefB and C systems, and resulted in a decrease of intracellular pH (Ferguson et al., 1995). In KefB and C deletion mutants the decrease of intracellular pH was much less than in strain harboring the efflux systems (Ferguson et al., 1995). KefB and C were shown to be activated by S-D-lactoylglutathione and to a less extent by hemithioacetal adduct of glutathione and methylglyoxal, and inhibited by glutathione itself (Ferguson et al., 1993; MacLean et al., 1998). The degree of homology of amino acid sequences of different glutathione utilizing enzymes is a subject of debate, therefore it is interesting to note that KefC shows sequence similarity to *Pseudomonas putida* glyoxalase I only between M4 and M5 putative transmembrane helices (McKie and Douglas, 1993). The acidification of intracellular matrix by acetic acid protected cells against methylglyoxal provoked injury (Ferguson et al., 1995). Hence, the absence of acidification for whatever reasons led to the drop of viability (Ferguson et al., 1995). While an active Kef system protected cells from electrophile attack, the high affinity K^+ uptake system (Kdp) sensitized cells to any injury caused by methylglyoxal as the activation of Kdp counteracts the acidification process directed by Kef system (Ferguson et al., 1995, 1996). Furthermore, generating a wide variety of other mutants it turned out that in *E. coli* (i) similarly to other living organisms the main methylglyoxal detoxifying route operates through glyoxalases; (ii) glyoxalase I is not needed for the restoration of normal $[K^+]_{IC}$; (iii) the increase of intracellular pH is protective to the cells even in the lack of glutathione but does not stimulate the detoxication of the aldehyde; (iv) glutathione plays a pivotal role in the defense against the attack by methylglyoxal as a cofactor for glyoxalases (Ferguson et al., 1995, 1996; Ferguson and Booth, 1998; MacLean et al., 1998). The role of glutathione in the protection against methylglyoxal is also emphasized by the work of Murata et al. (1980) who constructed a methylglyoxal resistant mutant secreting glutathione into the medium. The mutant had high activities for glutathione producing enzymes and for the glyoxalases setting out the important role of both glutathione and glyoxalases in the protection of cells against this aldehyde (Murata et al., 1980). It is to be noticed that methylglyoxal stimulated glutathione secretion of the mutant by about 50-fold (Murata et al., 1980). Taking together their data Boots et al. asserted two lines of protection from methylglyoxal toxicity. The direct mechanism operates through glyoxalase pathway thus detoxifying the electrophile, while an indirect mechanism is also operative in which the acidification of

intracellular space takes place (Ferguson et al., 1995). The glutathione adducts of methylglyoxal, most effectively S-D-lactoylglutathione, function as signals for the Kef system and the activated ion channels mediate a potassium efflux linked to proton uptake by the cells (Ferguson et al., 1995). From this point of view the existence of Kdp system counteracting the function of Kefs, is disadvantageous to the cells in the regard of survival (Ferguson et al., 1996). In addition, the glyoxalase I and II would have a critical role in defense not only by detoxifying methylglyoxal but also by regenerating intracellular glutathione (Ferguson and Booth, 1998). Nevertheless, the mode of protective action of pH lowering is unclear.

Although the works discussed above have a high impact in the understanding of the mechanisms of protection against methylglyoxal toxicity, indeed there are points which have to be critically examined. Though it is beyond doubt that glyoxalases may be implicated in the regeneration of intracellular GSH, observations gained on hepatocytes exposed to methylglyoxal do not support the flat opinion presented above (Kalapos et al., 1992a; Ferguson and Booth, 1998). It was shown that in hepatocytes incubated in nutriments supplemented Krebs–Henseleit buffer methylglyoxal caused only a transient fall of cellular GSH level and the recovery of normal intracellular glutathione concentration was mainly due to de novo synthesis of tripeptide (Kalapos et al., 1992a). Maybe it is not premature to consider that the situation in procaryotes is, at least in part, similar to that in mammalian cells, eventhough soberness is needed while interpreting data. It is also troublesome to accept the proposal whether chemicals exerting inhibitory effect on either methylglyoxal synthase or glyoxalase I may be used as antibacterial agents in clinical practice (MacLean et al., 1998; Tötemeyer et al., 1998). It is known that glyoxalase inhibitors have been offered as anticancer compounds (Vince and Wadd, 1969), but to date neither of them have been introduced as medicine because in animal experiments there has been a bad variation in their effectivity (Kalapos, 1999a). Admittedly, it is not clear yet how to fulfill the criterium of selective toxicity against bacterial cells as the ability of methylglyoxal to initiate cell death is not restricted to procaryotic cells (Braun et al., 1994).

Despite uncertainties the findings published upon procaryotic systems make possible to outline a scennario about how methylglyoxal metabolism fits to biochemical network. Clearly, all the conditions resulting in an excessive surfeit of methylglyoxal are associated with a loss of control over carbohydrate metabolism (Ferguson et al., 1995; Russell and Cook, 1995; Ferguson et al., 1996; MacLean et al., 1998; Tötemeyer et al., 1998). Bacterial cells accumulate methylglyoxal when (i) the flux in glycolytic sequence is higher than that needed for growing or (ii) the flux through the transport systems is elevated thus overburdening the upper part of glycolysis. This latter happens either in mutants being unable to control their transport proteins or in those cases when there is a shift from the low carbon source cultivation, other than glucose, to high carbon source condition as a consequence of the maintained induction of catabolite repressed operons by the failure to downregulate cAMP pools. In this scenario the provision of P_i and detoxication of methylglyoxal are related to energy spilling thus enhancing to balance metabolism. The only problem with this picture is that the non-enzymatic methylglyoxal formation is not taken into account (for details see Section 3.3).

4.3. Methylglyoxal toxicity in vitro

4.3.1. Methylglyoxal and cellular energy production in mammals

Glucose formation from methylglyoxal was described in 1984 (Casazza et al., 1984). Three phases for glucose production from methylglyoxal were demonstrated; at concentrations up to 1 mM, glucose formation from the aldehyde increased as its concentrations elevated, between 1 and 5 mM concentrations a plateau was observed and at concentrations higher than 5 mM, glucose synthesis fell (Kalapos et al., 1991b). In isolated murine hepatocytes both the character and the rate of glucose formation were independent of whether the animals from which the cells originated, were pretreated or not; the phenomenon was the same in all the cases (Kalapos et al.,

1991b, 1994, 1996a). Interestingly, concentrations of methylglyoxal above 1 mM led to an inhibition of protein synthesis (Kalapos et al., 1991b). Generally accepted is the fact that glucose synthesis from this aldehyde happens via pyruvate. But noteworthy is that when the cells were incubated in the presence of monoiodo acetic acid, an inhibitor of glyceraldehyde 3-phosphate dehydrogenase, glucose production was only decreased but not prevented from methylglyoxal while it was fully abolished if alanine was the substrate (Riba et al., 1992). The interpretation of this observation was that a pathway operating without pyruvate, may also exist (Riba et al., 1992).

Although the regulation of glucose production from methylglyoxal has not yet been investigated thoroughly, there are observations on its positive calcium-dependent regulation (Kalapos et al., 1992b). In addition, gluconeogenesis from methylglyoxal was increased by insulin in a concentration dependent way in hepatocytes prepared from overnight starved streptozotocin-induced diabetic mice (Kalapos et al., 1996a). This phenomenon was not seen in the case of pyruvate (Kalapos et al., 1996a).

Further support to gluconeogenesis was gained while examining the relationship between methylglyoxal and drug oxidation. The shape of accumulation curves for oxidized drugs in isolated mouse hepatocytes changed parallel with that of glucose formation irrespective of the pretreatment of animals prior to the experiments (Kalapos et al., 1991b, 1996a). As expected, the measure of glucose generation was lowered under these circumstances reflecting the fact that the NADPH + H^+ supply for cytochrome P450s, which catalyze the oxidation of drugs, by diverting intermediates of gluconeogenic sequence burdens gluconeogenesis (Kalapos et al., 1991b). The conversion of acetone, an endogenous substrate for cytochrome P450 IIE1 gene products, was also enhanced providing an additional and interesting evidence of this interrelationship (Kalapos et al., 1996b).

Methylglyoxal inhibited glucose utilization as well as lactate formation in both Ehrlich ascites tumour cells and in human platelets whereas the platelet glycogen breakdown and lactate production in normal human leucocytes were not affected by the aldehyde (Leoncini et al., 1989; Halder et al., 1993; Bitwas et al., 1997). Nevertheless, the results concerning the inhibition of glycolytic enzymes are quite contradictory as e.g. in one case glyceraldehyde 3-phosphate dehydrogenase activity was inhibited while in another case was not affected (Leoncini et al., 1989; Halder et al., 1993). Contrasted behaviour of other enzymes from various sources or in different experiments has already been pointed out though the answer to the problem is not known yet [Kalapos (1994a) and references therein].

In 1932, Kisch described the selective increasing effect of methylglyoxal on the respiration of cardiac tissue (Kisch, 1932). The O_2 consumption of other tissues involving Jensen-sarcoma cells was decreased by methylglyoxal (Kisch, 1932). In the 1990s, the Rays et al. investigated the phenomenon mentioned above. They found that methylglyoxal diminished mitochondrial respiration in mitochondria originated from tumour tissues in an irreversible manner, while the aldehyde was without any effect when mitochondria were prepared from normal tissues (Ray et al., 1991, 1994; Bitwas et al., 1997). The only exception from the rule was the cardiac tissue since the oxygen uptake of mitochondria isolated from the heart of various species was decreased by methylglyoxal (Ray et al., 1997). Similarly to its effect on mitochondrial respiration, methylglyoxal decreased oxygen consumption of cells and tissue slices originating from cancerous tissues whereas it had no effect on that in case of normal counterparts (Ray et al., 1991). Interestingly, L-lactaldehyde exerted a concentration dependent protection against the inhibitory effect of methylglyoxal in all the cases (Ray et al., 1991, 1994, 1997). Using specific inhibitors and effectors of respiratory chain proteins complex I was suggested to be the target of the action of methylglyoxal (Ray et al., 1994; Bitwas et al., 1997; Ray et al., 1997). In the lack of direct evidence for the binding of methylglyoxal to complex I of the respiratory chain it is troublesome to say that the site of methylglyoxal provoked inhibition on NAD^+ related respiration is surely the complex I. As on the basis of findings we are only aware of an inhibition due to the deactivation of any step

being between NADH + H^+ production and the transfer of electrons to ubiquinone.

The findings on the behaviour of cardiac tissue in the regard of oxygen consumption are contradictory (Kisch, 1932; Ray et al., 1997). The confusion is further increased if that fact is taken into account according to which the malate dehydrogenase from pig heart is not a subject of methylglyoxal inhibition while the enzyme from other sources is fairly inactivated by the aldehyde (Kalapos, 1994a). Again, as yet it is not clear to what this difference ought to be attributed.

The addition of methylglyoxal led to the depletion of ATP in Ehrlich ascites cells and in human leukaemic leucocytes, as well as in human platelets, but not in human normal leucocytes (Leoncini et al., 1989; Halder et al., 1993; Bitwas et al., 1997). The loss of intracellular ATP can be a consequence of the inhibition of glycolysis and respiration. The change of ATP content of cardiac tissue after addition of methylglyoxal has not been investigated yet.

Based on their results gained with normal and cancerous tissues the Rays raised a hypothesis according to which the excessive ATP formation may transform normal cells into malignant as the overwhelming amount of ATP may function as a trigger for anabolic reactions and cell growth (Ray et al., 1997). The continuous work of heart makes possible the maintenance of balance between ATP production and breakdown in cardiac tissue thus avoiding malignant transformation (Ray et al., 1997). Eventhough the theory is attractive it is not clear why the sensitivity to methylglyoxal of complex I of tumour tissue and that of cardiac cells ought to be similar since the utilization of energy in these cases is quite different, namely energy spilling and mechanic work, respectively. Perhaps the isolation and sequence analysis of complex I proteins may answer the question.

4.3.2. Generation of reactive oxygen species

The mutagenicity of methylglyoxal and the modulation of its mutagenic action by different agents have been known for a long time [for review see Sugimura and Sato (1983)]. Nevertheless, only in 1983 demonstrated Yamaguchi and Nakagawa that the mutagenic ability of methylglyoxal and trioses depended on the presence of oxygen radicals generated in the reactions (Yamaguchi and Nakagawa, 1983). The addition of scavangers to the media revealed that superoxide anion and hydrogen peroxide were produced as reactive oxygen derivatives (Yamaguchi and Nakagawa, 1983). The autoxidation of methylglyoxal and its ability to generate reactive oxygen species have also been proven by the measurement of oxygen consumption as well as by ferricytochrome c reduction assay (Thornalley et al., 1984). Unfortunatelly, neither the carbon product nor the oxygen species have been identified in these cases (Thornalley et al., 1984). In this regard, it is needed to note that in the mid-1930s a slow oxidation of methylglyoxal to carbon dioxide by air was already published (Spoehr and Milner, 1934). Yet, it should also be noticed that the sensitivity of methylglyoxal to autoxidation was concordantly reported to be less than that of dihydroxiacetone, glyceraldehyde or glucose (Spoehr and Milner, 1934; Thornalley et al., 1984).

Similar to the glycation of proteins by monosaccharides, under aerobic conditions, the generation of superoxide anion has been documented during the glycation reaction of amino acids by methylglyoxal (Yim et al., 1995). Beside oxygen derived free radical two other kinds of free radicals were also generated: the cross-linked radical cation and the methylglyoxal radical anion (Yim et al., 1995). However, in the absence of oxygen only the last two types of radicals were present showing the oxygen insensitivity of protein crosslinking (Yim et al., 1995). As superoxide anion initiates lipid peroxidation (Gutteridge and Halliwell, 1990), it may also be a contributing factor to lipid peroxidation documented in animals treated with methylglyoxal (Choudhary et al., 1997).

In a cellular system, the production of reactive oxygen species was only described in 1993 (Kalapos et al., 1993). Using cultured rat hepatocytes a dose-dependent effect of methylglyoxal on oxygen derived free radical production was seen; mainly H_2O_2 was detected (Kalapos et al., 1993). Of course, the release of reactive oxygen was not

detectable under unaerobic conditions, eventhough methylglyoxal afforded its toxicity to the cells (Kalapos et al., 1993). Again, reactive oxygen species were not essentially involved in methylglyoxal related toxicity. This notion is further supported by the fact that the release of reactive oxygen only became significant when a large portion of hepatocytes had already lost their viability (Kalapos et al., 1993). In human platelets, methylglyoxal, when added alone, resulted in a slight increase of hydrogen peroxide level, while administered together with thrombin, a marked accumulation of H_2O_2 was shown (Leoncini and Poggi, 1996). Parallel to the above mentioned event, modification of platelet functions, such as inhibition of platelet aggregation or ATP release induced by thrombin, were also demonstrated (Leoncini and Poggi, 1996). In rat aortic smooth muscle cells, an elevation of heparin-binding epidermal growth factor (HB-EGF)-like growth factor mRNA level was caused by methylglyoxal in a time- and dose-dependent manner (Che et al., 1997). The induction of gene expression was linked to the elevation of intracellular hydrogen peroxide concentration (Che et al., 1997). The incubation of cells with H_2O_2 also increased the mRNA level of HB-EGF-like growth factor indicating the involvement of reactive oxygen species in the process (Che et al., 1997). None the less, in the lack of data that would have been gained from the experiments undertaken under anaerobic conditions, it is not clear whether the induction of gene expression in the above special case can be managed in the absence of oxygen, as well. The question is not a nonsense question as methylglyoxal has been reported to inhibit the in vitro histone acetylation in cell-free extract of rat uterus (Procaccini et al., 1971). Hence, its interaction with any of the proteins involved in gene expression cannot be excluded ab ovo.

In summary, these findings show a relationship between the actions of methylglyoxal and its ability to generate reactive oxygen species whereas, as it now looks, the release of oxygen derived free radicals is not a prerequisite of methylglyoxal toxicity but seems to be needed when the storage or the expression of genetic information are somehow influenced by this α-oxoaldehyde.

4.3.3. Cell killing and methylglyoxal

The cytotoxicity of methylglyoxal has already been shown in this paper, therefore here only its role in the programmed cell death (apoptosis) is mentioned. The addition of methylglyoxal to different cell lines resulted in nuclear fragmentation and apoptotic body formation indicating an increase of apoptosis (Okado et al., 1996). Butionine sulfoximine, a potent inhibitor of glutathione synthesis, enhanced apoptosis whereby it was suggested that an intracellular oxidative stress was the cause of methylglyoxal induced apoptosis (Okado et al., 1996).

5. Biological implications

5.1. Clinical significance of methylglyoxal metabolism in diabetes mellitus

Although insulin dependent diabetes mellitus (IDDM) shows association to HLA, no significant association of glyoxalase I alleles, also located to chromosome 6 in humans, with IDDM has been recognized (Moens et al., 1980; McCann et al., 1981; Tokunaga et al., 1982). In the case of Caucasian IDDM patients a significant increase in $GLO^{1\text{-}1}$ has been reported, while in non-insulin dependent diabetic patients (NIDDM) the disturbance of distribution of glyoxalase types has not been seen (McCann et al., 1981). In another series of experiments just the opposite situation has been found, a significant disturbance in the percent gene frequencies with an excess of GLO^2 allele and a lack of any change in allele frequencies have been described in NIDDM and IDDM, respectively (Kirk et al., 1979). Despite the contradictions, nowadays it looks, no association between the incidence of diabetes mellitus and the distribution of glyoxalase genotypes can be verified. On the contrary, glyoxalase genotypes are in a link to the development of diabetic complications as shown by the disturbance in the distribution of glyoxalase alleles related to the presence or the absence of diabetic complications (McCann et al., 1981).

There are several other observations which are suggestive of a role of methylglyoxal in the devel-

opment of diabetic complications. The overexpression of glyoxalase I hindered the formation of advanced glycation end-products (Shinohara et al., 1996), the addition of aldose reductase inhibitors to streptozotocin-induced diabetic rats prevented the increase of methylglyoxal levels in kidney, lens and blood, and the glyoxalase I activities were in a correlation with the occurance of diabetic complications (Thornalley, 1994). And some other similarities between diabetic state and methylglyoxal treatment of animals have already been noted (Sections 3.1, 3.3 and 4.2).

Almost at the same time two papers appeared trying to integrate experimental data on methylglyoxal into a theory explaining the contribution of the aldehyde to the development of diabetic complications (Vander Jagt et al., 1992; Kalapos, 1992). In one of them the role of aldose reductase was highlighted, while in the other one the depression of glutathione stores and the subsequently emerging oxidative pressure were thought to be the most important pathological factors (Vander Jagt et al., 1992; Kalapos, 1992). The modification of proteins as a consequence of the action of dicarbonyl was involved in both theories (Vander Jagt et al., 1992; Kalapos, 1992). The integration of these hypotheses into one concept probably describes the real situation much better. Since very recently papers on the possible roles of dicarbonyls and on a new aspect of acetone metabolism have appeared (Baynes and Thorpe, 1999; Kalapos, 1999b), further discussion of this topic is avoided here, and only some problems not having been addressed until now are mentioned. What is the source of methylglyoxal in different tissues? In what extent can ketone body degradation contribute to methylglyoxal production, particularly reminding of the induction of cytochrome P450 IIE1 gene isozymes and non-enzymatic conversion of acetoacetate into methylglyoxal? As in diabetic ketosis ketone body formation exceeds the degrading capacity and the accumulating acetoacetate presents a challenge to the pH regulatory system, the question is how ketotic and non-ketotic states differ from metabolic point of view? Finally, what kind of therapy on the basis of recent data can be worked out?

5.2. Cancer paradox

Methylglyoxal has been shown to induce tumorigenesis, but it has been reported to be cancerostatic, too (Section 4.2). Although the situation is a bit paradoxical it can be explained if the action of methylglyoxal on DNA is investigated. As reported, double stranded DNA was protected against while single stranded DNA was susceptible to the attack of methylglyoxal (Krymkiewicz, 1973). Both the formation of methylglyoxal-guanine adduct and a competition of aldehyde with nucleotides for hydrogen bonding have been suggested as possible mechanisms for the interaction of methylglyoxal with DNA [for review see Kalapos (1994a)]. At the same time, the cancerostatic action of methylglyoxal simply comes from its reactive nature, that is when methylglyoxal burden exceeds detoxifying capacity of a given cell, the aldehyde immediately exerts its cell killing effect. Since the capacity of glyoxalase pathway in various cancerous and non-tumour tissues is seen to be very different this is one of the reasons of why methylglyoxal or its derivatives cannot be used successfully for the treatment of cancer.

5.3. Possible roles in other diseases

Seventy years ago the similarities between the symptoms caused by methyglyoxal administration and those displayed by animals suffering in B1-avitaminosis (beri-beri) led to the suggestion that beri-beri was a methylglyoxal intoxication (Vogt-Moller, 1929; Salem, 1954). Since then a quantity of data have been gathered and though in essence our knowledge is not enough to accept or refuse the idea emerged, nowadays it does not seem likely that beriberi is a methylglyoxal intoxication [review see Kalapos (1994a)]. But rather the heaping of dicarbonyl is a result of disturbed metabolism through a yet unknown mechanism.

Another disease being thought to be related to methylglyoxal metabolism is the encephalopathy in short-bowell syndrome, in which D-lactate has been suggested as a pathogenic agent (Kalapos, 1994b). The clinical basis of this suggestion was the occurance of D-lactate in the plasma of pa-

tients (Oh et al., 1979; Ayub et al., 1981). Most serious criticism to D-lactate as a cause of disease is that neither the severity of symptoms correlated with the measure of D-lactate concentration in the blood, nor the symptoms were provoked when healthy patients were infused with high concentrations of acid (Kalapos, 1994b). Therefore, it has been suggested that methylglyoxal is the real agent leading to the development of these symptoms (Kalapos, 1994b). Supporting observations are: (i) D-lactate is a product of the transformation of methylglyoxal; (ii) methylglyoxal interacts with biogenic amines in vitro (Szent-Györgyi and McLaughlin, 1975); (iii) in experiments with nervous tissues a depolarizing effect of the aldehyde has been revealed (Chambers et al., 1985; Davies et al., 1986). Spite of above listed facts further experimental data are required to clear the situation.

5.4. Theories on the biological function(s) of methylglyoxalase pathway

The most influential theories raised over the decades are as follows: (i) participation in glycolysis; (ii) regulation of cell division (promine and retine) and (iii) detoxication of methylglyoxal. Nevertheless, either of those have proven to give an acceptable answer to the long lasting problem concerning the function of this pathway. (i) The Embden–Meyerhof scheme of glucose breakdown operates through another route (Meyerhof, 1948). (ii) The theory of Szent-Györgyi suffers from intramural contradictions (Kalapos, 1999a). The most serious concerns making the theory questionable are that neither the intracellular concentration of methylglyoxal nor the glyoxalase activities display clear cell cycle dependent changes during cell division, and cancerous tissues and their normal counterparts do not show the relationship between methylglyoxal concentrations and glyoxalase activities that might be expected (Kalapos, 1999a). In addition, compartmentation for glyoxalases is not seen, and promine and retine have never been isolated and identified as glyoxalase and methylglyoxal, respectively (Kalapos, 1999a). (iii) Although detoxication exactly describes a function for glyoxalases, which probably may be one of the functions in extant metabolism, the concept does not give the reason of why the pathway is of ubiquitous nature (Aronsson and Mannervik, 1977).

Recently a new suggestion has been raised. According to that the function of methyl-glyoxalase path has to be traced back to the area of prebiotic evolution by supposing that the pathway might have served as an anaplerotic route for reductive citric acid cycle in surface metabolists (Kalapos, 1997). The raw molecule for the network would have been formaldehyde (Kalapos, 1999c). The advantage of this hypothesis is that the emergence of energy rich bonds arises from the concept in a plausible manner and the proposed function does not counter with the detoxication concept (Kalapos, 1998).

6. Conclusion and perspectives

As shown in this paper, living organisms have the ability to produce methylglyoxal (Section 3). If once it is formed there should be at least one enzyme eliminating it because of its high reactivity and bad toxicity (Section 2–Section 4). If we followed the conventions it would be possible to end up our discussion here by saying glyoxalases do the detoxification of methylglyoxal and that is why those are widespread. Into this picture it would be easy to fit all the toxic events caused by methylglyoxal either in vivo or in vitro.

However, the above notion may be questioned and needs to be surveyed. First of all, eventhough glyoxalases are widespread there are species lacking glyoxalase I (see *Cestodes and Digeneans* in chapter Section 4.2). Furthermore, if we are attentive to the facts it becomes obvious that glyoxalase II deficiency in red blood cells of either horse or human being may be circumvented (Valentine et al., 1970; Agar et al., 1984). If we accepted the suggestion that the only role of glyoxalases would be to detoxify methylglyoxal being produced as an offshoot of glycolysis then the immediate question would arise why there are so many enzymes being involved in methylglyoxal production (Sections 3.1 and 3.2).

As seen in evolution, Nature does not indulge in luxuries and she does not throw away a mechanism when proved successful. Therefore, an important role had to be assigned to this system in the course of evolution. As recently raised, methylglyoxalase pathway might have had an anaplerotic route for citric acid cycle in the early stage of evolution (Kalapos, 1997). This view has the advantage over others since in this case the network already fits to carbohydrate metabolism at the early stage of evolution and hence it is not a surprise that in the extant metabolism it is still ultimately bound to glycolysis. Of course, its metabolic significance changed over the evolutionary process but under pathological conditions its subordinate role becomes essential, e.g. in diabetes mellitus when the elimination of α-oxoaldehydes is of vital importance. Thus the evolution of this network exemplifies how Nature preserves a function under changing circumstances.

Taken together, it is assumed that the role of glyoxalase pathway, from methylglyoxal synthase to D-lactate dehydrogenase in the extant metabolism, is to detoxify methylglyoxal which is unavoidably produced in glycolytic bypass. While in the early state of metabolic development it might have functioned as an anaplerotic route for citric acid cycle.

From clinical point of view further research may be predicted towards the investigation of the possible role of α-oxoaldehydes in the development of diabetic complications and also in other diseases where glycation has been suggested to play a role. Thereby there is a hope for a better understanding of pathological events. In this regard the examination of toxic effects of methylglyoxal is essential. And the description of fine elements of the mode of action of enzymes involved in methylglyoxal household and the bioengineering work on those are very probable. However, successful application of glyoxalase I inhibitors as antitumour and antibacterial agents may not be expected.

Acknowledgements

The author is indebted to Etelka Imre B.Sc. (Theoretical Biology Research Group) and Pál Riba M.D. (Semmelweis University, Medical School) for their help in collecting the literature. The publication of this paper was made possible by the grant from the National Scientific Research Found (OTKA), Budapest, Hungary (T 022914).

References

Ackerman, R.S., Cozzarelli, N.R., Epstein, W., 1974. Accumulation of toxic concentrations of methylglyoxal by wild-type *Escherichia coli* K-12. J. Bact. 119, 357–362.

Agar, N.S., Board, P.G., Bell, K., 1984. Studies of erythrocyte glyoxalase II in various domestic species: discovery of glyoxalase II deficiency in the horse. Anim. Blood Group Biochem. Genet. 15, 67–70.

Ahmed, M.U., Brinkmann Frye, E., Degenhardt, T.P., Thorpe, S.R., Baynes, J.W., 1997. N^{ε}-(Carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem. J. 224, 565–570.

Apple, M.A., Greenberg, D.M., 1967. Arrest of cancer in mice by therapy with normal metabolites. Cancer Chemother. Rep. 51, 455–464.

Ariyama, N., 1928. Studies on glyoxals. J. Biol. Chem. 77, 359–394.

Aronsson, A.C., Mannervik, B., 1977. Characterization of glyoxalase I purified from pig erythrocytes by affinity chromatography. Biochem. J. 165, 503–509.

Ashwood-Smith, M.J., Robinson, D.M., Barnes, J.H., Bridges, B.A., 1967. Radiosensitization of bacterial and mammalian cells by substituted glyoxals. Nature Lond. 216, 137–139.

Atkinson, R., Carter, W.P.L., Darnall, K.R., Winer, A.M., Pitts, J.N., 1980. A smog chamber and modelling study of gas phase NO_x-Air photooxidation of toluene and the cresols. Int. J. Chem. Kinetics 12, 779–836.

Ayub, A., Falcon, W.W., Heinig, R.E., 1981. Encephalopathy following jejunoileostomy. J. Am. Med. Assoc. 246, 970–973.

Baggetto, L.G., Lehninger, A.L., 1987. Isolated tumoral pyruvate dehydrogenase can synthesize acetoin which inhibits pyruvate oxidation as well as other aldehydes. Biochem. Biophys. Res. Commun. 145, 153–159.

Barrenscheen, H.K., Braun, K., 1931. Farb- und Fallungsreaktionen des Methylglyoxals. Biochem. Zeitschr. 233, 296–304.

Barrenscheen, H.K., Dreguss, M., 1931. Eine kolorometrische Mikromethode zur Bestimmung des Methylglyoxals. Biochem. Zeitschr. 233, 305–310.

Barrenscheen, H.K., 1928. Methylglyoxal als intermediares Spaltungsprodukt bei der Glykolyse des Blutes. Biochem. Zeitschr. 193, 105–108.

Baumann, E., 1885. Über Verbindungen der Aldehyde, Ketone und Ketosauren mit den Merkapten. Ber. Dtsch. Chem. Gesells. 18, 883–892.

Baynes, J.W., Thorpe, S.R., 1999. Role of oxidative stress in diabetic complications. Diabetes 48, 1–9.

Bender, K., Seibert, R.T., Weinker, T.F., Kren, V., Pravenec, M., Bissbort, S., 1994. Biochemical genetics of methylglyoxal reductase in the laboratory rat (*Rattus norvegicus*). Biochem. Genet. 32, 147–154.

Bernhauer, K., Görlich, B., 1929. Über Zuckeroxydationen und -zersetzungen. Biochem. Zeitschr. 212, 452–465.

Bitwas, S., Ray, M., Misra, S., Dutta, D.P., Ray, S., 1997. Selective inhibition of mitochondrial respiration and glycolysis in human leukaemic leucocytes by methylglyoxal. Biochem. J. 323, 343–348.

Bloch, H., Lehr, H., Erlenmeyer, H., und Vogler, K., 1945. Der Einfluss von 1,2-Diketonen auf das Wachstum von Tuberkelbazillen. Helv. Chim. Acta 28, 1410–1413.

Bonsignore, A., Leoncini, G., Siri, A., Ricci, D., 1970. The lysine catalysed reaction transforming glyceraldehyde into methylglyoxal. Ital. J. Biol. Chem. 19, 284–301.

Bonsignore, A., Leoncini, G., Siri, A., Ricci, D., 1973. Kinetic behaviour of glyceraldehyde 3-phosphate conversion into methylglyoxal. Ital. J. Biochem. 22, 131–140.

Braun, L., Garzó, T., Riba, P., Mandl, J., Kalapos, M.P., 1994. Methylglyoxal and cell viabitily. Int. J. Biochem. 26, 987–990.

Brennan, M., 1989. Changes in solubility, non-enzymatic glycation, and fluorescence of collagen in tail tendons from diabetic rats. J. Biol. Chem. 264, 20947–20952.

Bronzetti, G., Corsi, C., Del Chiario, D., Boccardo, P., Velosi, R., Rossi, F., Paolini, M., Cantelli Forti, G., 1987. Methylglyoxal: genotoxicity and its effects in vivo on the hepatic microsomal mono-oxygenase system of the mouse. Mutagenesis 2, 275–277.

Brophy, P.M., Crowley, P., Barrett, J., 1990. Relative distribution of glutathione transferase, glyoxalase I and glyoxalase II in helmints. Int. J. Parasitol. 20, 259–261.

Brum, V.C., 1966. Synthesis of methylglyoxal-^{14}C. J. Pharmaceut. Sci. 55, 351–352.

Burke, R.M., Tempest, D.W., 1990. Growth of *Bacillus stearothermophilus* on glycerol in chemostat culture: expression of an unusual phenotype. J. Gen. Microbiol. 136, 1381–1385.

Carter, W.P.L., Lloyd, A.C., Sprung, J.L., Pitts, J.N., 1979. Computer modelling of smog chamber data: progress in validation of a detailed mechanism for the photooxidation of propene and n-butane in photochemical smog. Int. J. Chem. Kinetics 11, 45–101.

Casazza, J.P., Felver, M.E., Veech, R.L., 1984. Metabolism of acetone in rat. J. Biol. Chem. 259, 231–236.

Case, E.M., Cook, R.P., 1931. The occurance of pyruvic acid and methylglyoxal in muscle metabolism. Biochem. J. 25, 1319–1335.

Cazzulo, J.J., 1992. Aerobic fermentation of glucose by trypanosomatids. FASEB J. 6, 3153–3161.

Chambers, P.L., Davies, M.G., Rowan, M.J., 1985. The effects of methylglyoxal on central synaptic transmission in the isolated nerve cord of the Cockroach (*Periplanta Americana* L). Arch. Toxicol. Suppl. 8, 337–341.

Chaplen, F.W.R., Fahl, W.E., Cameron, D.C., 1996. Detection of methylglyoxal as a degradation product of DNA and nucleic acid components treated with strong acid. Anal. Biochem. 235, 262–269.

Che, W., Asahi, M., Takahashi, M., Kaneto, H., Okado, A., Higashiyama, S., Taniguchi, N., 1997. Selective induction of heparin-binding epidermal growth factor-like growth factor by methylglyoxal and 3-deoxyglucosone in rat aortic smooth muscle cells. J. Biol. Chem. 272, 18453–18459.

Choudhary, D., Chandra, D., Kale, R.K., 1997. Influence of methylglyoxal on antioxidant enzymes and oxidative damage. Toxicol. Lett. 93, 141–152.

Clelland, J.D., Thornalley, P.J., 1990. Synthesis of ^{14}C-labelled methylglyoxal and S-D-lactoylglutathione. J. Labelled Comp. Radiopharm. 28, 1455–1464.

Coleman, D.L., 1980. Acetone metabolism in mice: Increased activity in mice heterozygous for obesity genes. Proc. Natl. Acad. Sci. USA 77, 290–293.

Conroy, P.J., 1978. Carcinostatic activity of methylglyoxal and related substances in tumour-bearing mice. Ciba Found. Symp. 67, 271–300.

Cooper, R.A., 1975. Methylglyoxal synthase. Methods Enzymol. 41, 502–508.

Cooper, R.A., 1984. Metabolism of methylglyoxal in microorganisms. Ann. Rev. Microbiol. 38, 49–68.

Creighton, D.J., Migliorini, M., Pourmotabbed, T., Guha, M.K., 1988. Optimization of efficiency in the glyoxalase pathway. Biochemistry 27, 7376–7384.

Dakin, H.D., Dudley, H.W., 1913a. An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes. J. Biol. Chem. 14, 155–157.

Dakin, H.D., Dudley, H.W., 1913b. The interaction of alpha-amino acids, alpha-hydroxy acids and alpha-ketonic aldehydes. J. Biol. Chem. 15, 127–143.

Darling, T.N., Blum, J.J., 1988. D-lactate production by *Leishmania braziliensis* through the glyoxalase pathway. Mol. Biochem. Parasitol. 28, 121–128.

Darling, T.N., Balber, A.E., Blum, J.J., 1988. A comparative study of D-lactate, L-lactate and glycerol formation by four species of Leishmania and by *Trypanosoma lewisi* and *Trypanosoma brucei gambiense*. Mol. Biochem. Parasitol. 30, 253–258.

Davies, M.G., Chambers, P.L., Rowan, M.J., 1986. Effects of methylglyoxal on central and peripheral cholinergic responses. Arch. Toxicol. Suppl. 9, 46–50.

de V. Moulds, L., Riley, H.L., 1938. 'The polymerisation of methylglyoxal'. J. Chem. Soc. 621–626.

Deswal, R., Chakaravarty, T.N., Sopory, S.K., 1993. The glyoxalase system in higher plants: Regulation in growth and differentiation. Biochem. Soc. Trans. 21, 527–530.

Dianzani, M.U., 1978. Biological activity of methylglyoxal and related aldehydes. Ciba Found. Symp. 67, 245–270.

Douglas, K.T., Quilter, A.J., Shinkai, S., Ueda, K., 1985. Trapping of reactive intermediates in enzymology. Exogenous flavin reduction during catalytic turnover of substrate by glyoxalase I. Biochim. Biophys. Acta 829, 109–119.

Együd, L.G., Szent-Györgyi, A., 1966a. Cell division, SH, ketoaldehydes, and cancer. Proc. Natl. Acad. Sci. USA 55, 388–393.

Együd, L.G., Szent-Györgyi, A., 1966b. On the regulation of cell division. Proc. Natl. Acad. Sci. USA 56, 203–207.

Elliott, W.H., 1959. Amino-acetone: its isolation and role in metabolism. Nature (London) 183, 1051–1052.

Elliott, W.H., 1960. Methylglyoxal formation from aminoacetone by ox plasma. Nature (London) 185, 467–468.

Espartero, J., Sanchez-Aquayo, I., Pardo, J.M., 1995. Molecular characterization of glyoxalase-I from a higher plant: upregulation by stress. Plant Mol. Biol. 29, 1223–1233.

Fedoronko, M., Königstein, J., 1969. Kinetics of mutual isomerization of trioses and their dehydration to methylglyoxal. Collection Czechoslov. Chem. Commun. 34, 3881–3894.

Fedoronko, M., Königstein, J., 1971. Kinetik und Mechanismus der Disproportionierung von Methylglyoxal zu Milchsaure. Collection Czechoslov. Chem. Commun. 36, 3424–3430.

Ferguson, G.P., Booth, I.R., 1998. Importance of glutathione for growth and survival of *Escherichia coli* cells: Detoxification of methylglyoxal and maintenance of intracellular K^+. J. Bact. 180, 4313–4318.

Ferguson, G.P., Munro, A.W., Douglas, R.M., McLaggan, D., Booth, I.R., 1993. Activation of potassium channels during metabolite detoxification in *Escherichia coli*. Mol. Microbiol. 9, 1297–1303.

Ferguson, G.P., McLaggan, D., Booth, I.R., 1995. Potassium channel activation by glutathione-S-conjugates in *Escherichia coli*: protection against methylglyoxal is mediated by cytoplasmic acidification. Mol. Microbiol. 17, 1025–1033.

Ferguson, G.P., Chacko, A.D., Lee, C., Booth, I.R., 1996. The activity of the high-affinity K^+ uptake system Kdp sensitizes cells of *Escherichia coli* to methylglyoxal. J. Bact. 178, 3957–3961.

Freedberg, W.B., Kistler, W.S., Lin, E.C.C., 1971. Lethal synthesis of methylglyoxal by *Escherichia coli* during unregulated glycerol metabolism. J. Bact. 108, 137–144.

Friedemann, T.E., 1927. The action of alkali and hydrogen peroxide on glyoxals. J. Biol. Chem. 73, 331–335.

Fung, K., Grosjean, D., 1981. Determination of nanogram amounts of carbonyls as 2,4-dinitrophenylhydrazones by high-performance liquid chromatography. Anal. Chem. 53, 168–171.

Furihata, C., Sato, Y., Matsushima, T., Tatematsu, M., 1985. Induction of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by methylglyoxal. Carcinogenesis 6, 91–94.

George, S.E., Melton, T., 1986. Cloning and molecular characterization of csm mutations allowing expression of catabolite-repressible operons in the absence of exogenous cyclic AMP. J. Bact. 166, 533–540.

Ghoshal, K., Banerjee, A.B., Ray, S., 1989. Methylglyoxal-metabolizing enzymes of *Leishmania donovani* promastigotes. Mol. Biochem. Parasitol. 35, 21–30.

Gillespie, E., 1981. The tumor promoter phorbol ester, 12-o-tetradecanoylphorbol-13-acetate (TPA) increases glyoxalase I and decreases glyoxalase II activity in human polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. 98, 463–470.

Golej, J., Hoeger, H., Radner, W., Unfried, G., Lubec, G., 1998. Oral administration of methylglyoxal leads to kidney collagen accumulation in the mouse. Life Sci. 63, 801–807.

Gonzalez, F.J., 1989. The molecular biology of cytochrome P-450s. Pharmacol. Rev. 40, 244–288.

Grazi, E., Trombetta, G., 1978. A new intermediate of the aldolase reaction, the pyruvaldehyde-aldolase-orthophosphate complex. Biochem. J. 175, 361–365.

Griffith, C.E.F., Ong, L.H., Buettner, L., Creighton, D.J., 1983. Non-stereospecific substrate usage by glyoxalase I. Biochemistry 22, 2945–2951.

Gutteridge, J.M.C., Halliwell, B., 1990. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci. 15, 129–135.

Halder, J., Ray, M., Ray, S., 1993. Inhibition of glycolysis and mitochondrial respiration of Ehrlich ascites carcinoma cells by methylglyoxal. Int. J. Cancer 54, 443–449.

Harrison, J.E., Saeed, F.A., 1981. Acetoacetate is an electron donor to myeloperoxidase and a promoter of myeloperoxidase catalyzed fatty acid peroxidation. Biochem. Med. 26, 339–355.

Hasegawa, R., Ogiso, T., Imaida, K., Shirai, T., Ito, N., 1995. Analysis of the potential carcinogenicity of coffee and its related compunds in a medium term liver bioassay of rats. Fd. Chem. Toxic. 33, 15–20.

Hofman, K., 1953. Imidazole and its derivatives. Interscience Publishers. New York, pp. 33–35.

Hooke, A.M., Cerquetti, M.C., Zeligs, B.J., Wang, Z., Hoberg, K., Bellanti, J.A., 1993. Genetically stable temperature-sensitive mutants of *Salmonella typhi* induce protection in mice. Vaccine 11, 1386–1389.

Hopper, D.J., Cooper, R.A., 1971. The regulation of *Escherichia coli* methylglyoxal synthase; A new control of glycolysis? FEBS Lett. 13, 213–216.

Igawa, M., Munger, J.W., Hoffmann, M.R., 1989. Analysis of aldehydes in cloud- and fogwater samples by HPLC post-column reaction detector. Environ. Sci. Technol. 23, 556–561.

Inoue, Y., Kimura, A., 1995. Methylglyoxal and regulation of its metabolism in microorganisms. Adv.Microbial Phys. 37, 177–227.

Inoue, Y., Choi, B.Y., Murata, K., Kimura, A., 1990. Sexual response of *Saccharomyces cerevisiae*: Phosphorylation of yeast glyoxalase I by a cell extract of mating factor-treated cells. J. Biochem. (Tokyo) 108, 4–6.

Inoue, Y., Tran, L.T., Yoshikawa, K., Murata, K., Kimura, A., 1991. Purification and characterization of glyoxalase I from *Hansenula mrakii*. J. Ferment. Bioeng. 71, 131–133.

Inoue, Y., Ikemoto, S., Kitamura, K., Kimura, A., 1992. Occurence of a NADH-dependent methylglyoxal reducing system: Conversion of methylglyoxal to acetol by aldehyde reductase from *Hansenula mrakii*. J. Ferment. Bioeng. 74, 46–48.

Izaguirre, G., Kikonyogo, A., Pietruszko, R., 1998. Methylglyoxal as substrate and inhibitor of human aldehyde dehydrogenase: Comparison of kinetic properties among the three isozymes. Comp. Biochem. Physiol. 119B, 747–754.

Jerzykowski, T., Matuszewszki, W., Otrzonsek, N., Winter, R., 1970. Antineoplastic action of methylglyoxal. Neoplasma 17, 25–35.

Johnson, D.P., Critchfield, F.E., Ruch, J.E., 1962. Spectrophotometric determination of trace quantities of alpha-dicarbonyl- and quinone-type conjugated dicarbonyl compounds. Anal. Chem. 34, 1389–1391.

Kadner, R.J., Murphy, G.P., Stephens, C.M., 1992. Two mechanisms for growth inhibition by elevated transport of sugar phosphates in *Escherichia coli*. J. Gen. Microbiol. 138, 2007–2014.

Kalapos, M.P., Schaff, Z., Garzó, T., Antoni, F., Mandl, J., 1991a. Accumulation of phenols in isolated murine hepatocytes after pretreatment with methylglyoxal. Toxicol. Lett. 58, 181–191.

Kalapos, M.P., Garzó, T., Antoni, F., Mandl, J., 1991b. Effect of methylglyoxal on glucose formation, drug oxidation and glutathione content in isolated murine hepatocytes. Biochim. Biophys. Acta 1092, 284–290.

Kalapos, M.P., Garzó, T., Antoni, F., Mandl, J., 1992a. Accumulation of S-D-lactoylglutathione and transient decrease of glutathione level caused by methylglyoxal load in isolated hepatocytes. Biochim. Biophys. Acta 1135, 159–164.

Kalapos, M.P., Riba, P., Garzó, T., Antoni, F., Mandl, J., 1992b. The effect of A23187 on glucose production from methylglyoxal and pyruvate in isolated murine hepatocytes. Int. J. Biochem. 24, 411–414.

Kalapos, M.P., Littauer, A., de Groot, H., 1993. Has reactive oxygen a role in methylglyoxal toxicity? Arch. Toxicol. 67, 369–372.

Kalapos, M.P., Mandl, J., Bánhegyi, G., Antoni, F., Garzó, T., 1994. Net glucose production from acetone in isolated murine hepatocytes. The effect of different pretreatments of mice. Int. J. Biochem. 26, 1069–1079.

Kalapos, M.P., Riba, P., Garzó, T., Mandl, J., 1996a. Glucose formation from methylglyoxal in hepatocytes from streptozotocin-induced diabetic mice: the effect of insulin. Experientia 52, 827–830.

Kalapos, M.P., Riba, P., Garzó, T., Mandl, J., 1996b. Gluconeogenic precursors stimulate acetone metabolism in isolated murine hepatocytes. Int. J. Biochem. Cell Biol. 28, 705–709.

Kalapos, M.P., 1992. Mechanisms leading to complications in diabetes mellitus: Pathological role of α-oxoaldehydes. Biochem. Ed. 20, 27–29.

Kalapos, M.P., 1994a. Methylglyoxal toxicity in mammals. Toxicol. Lett. 73, 3–24.

Kalapos, M.P., 1994b. Can methylglyoxal be responsible for encephalopathy in short-bowel syndrome? Med. Sci. Res. 22, 387–388.

Kalapos, M.P., 1997. Possible evolutionary role of methylglyoxalase pathway/Anaplerotic route for reductive citric acid cycle of surface metabolists. J. Theor. Biol. 188, 201–206.

Kalapos, M.P., 1998. From mineral support to enzymatic catalysis/Further assumptions for the evolutionary history of glyoxalase system. J. Theor. Biol. 193, 91–98.

Kalapos, M.P., 1999a. On the promine/retine theory of cell division. Biochim. Biophys. Acta 1426, 1–16.

Kalapos, M.P., 1999b. Possible physiological roles of acetone metabolism in humans. Med. Hypotheses (in press).

Kalapos, M.P., 1999c. A possible evolutionary role of formaldehyde. Exp. Mol. Med. 31, 1–4.

Kato, R., Onoda, K.I., Takanaka, A., 1970. Species differences in drug metabolism by liver microsomes in Alloxan diabetic and fasted animals. Jpn. J. Pharmacol. 20, 546–553.

Kellum, M.W., Oray, B., Norton, S.J., 1978. A convenient quantitative synthesis of methylglyoxal for glyoxalase I assays. Anal. Biochem. 85, 586–590.

Kermack, W.O., Matheson, N.A., 1957. The effects of some analogous of glutathione on the glyoxalase system. Biochem. J. 65, 48–58.

Kersten, P.J., Kirk, T.K., 1987. Involvement of a new enzyme, glyoxal oxidase, in extracellular H_2O_2 production by *Phanerochaete chrysosporium*. J. Bact. 169, 2195–2201.

Kimura, A., Inoue, Y., 1993. Glyoxalase I in microorganisms: Molecular characteristics, genetics and biochemical regulation. Biochem. Soc. Trans. 21, 518–522.

Kirk, R.L., Theophilus, J., Whitehouse, S., Court, J., Zimmet, P., 1979. Genetic susceptibility of diabetes mellitus: the distribution of properdin factor B (Bf) and glyoxalase (GLO) phenotypes. Diabetes 28, 949–951.

Kisch, B., 1932. Beeinflussung der Gewebsatmung durch Methylglyoxal. Biochem. Zeitschr. 253, 373–376.

Knowles, J.R., 1991. Enzyme catalysis: not different, just better. Nature (London) 350, 121–124.

Koop, D.R., Casazza, J.P., 1985. Identification of ethanol-inducible P-450 isozyme 3a as the acetone and acetol monooxygenase of rabbit microsomes. J. Biol. Chem. 260, 13607–13612.

Krymkiewicz, N., Diéguez, E., Rekarte, U.D., Zwaig, N., 1971. Properties and mode of action of a bactericidal compound (= methylglyoxal) produced by a mutant *Escherichia coli*. J. Bact. 108, 1338–1347.

Krymkiewicz, N., 1973. Reactions of methylglyoxal with nucleic acids. FEBS Lett. 29, 51–54.

Leoncini, G., Poggi, M., 1996. Effects of methylglyoxal on platelet hydrogen peroxide accumulation, aggregation and release reaction. Cell Biochem. Funct. 14, 89–95.

Leoncini, G., Maresca, M., Buzzi, E., 1989. Inhibition of the glycolytic pathway by methylglyoxal in human platelets. Cell Biochem. Funct. 7, 65–70.

Leoncini, G., 1979. The role of alpha-oxoaldehydes in biological systems. Ital. J. Biochem. 28, 285–294.

Lieber, M.M., 1995. The role of cohesive fields in generating bud/plantlet developments from plant neoplasms. Front. Perspect. 4, 35–41.

Ly, H.D., Clugston, S.L., Sampson, P.B., Honek, J.F., 1998. Syntheses and kinetic evaluation of hydroxamate-based peptide inhibitors of glyoxalase I. Bioorg. Med. Chem. Lett. 8, 705–710.

Lyles, G.A., Chalmers, J., 1992. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem. Pharmacol. 43, 1409–1414.

Lyles, G.A., 1996. Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: Biochemical, pharmacological and toxicological aspects. Int. J. Biochem. Cell Biol. 28, 259–274.

MacLean, M.J., Ness, L.S., Ferguson, G.P., Booth, I.R., 1998. The role of glyoxalase I in the detoxication of methylglyoxal and in the activation of the KefB K^+ efflux system in *Escherichia coli*. Mol. Microbiol. 27, 563–571.

Mankikar, S., Rangekar, P., 1974. Effects of methylglyoxal on germination of barley. Φyton 32, 9–16.

Mannervik, B., Ridderström, M., 1993. Catalytic and molecular properties of glyoxalase I. Biochem. Soc. Trans. 21, 515–517.

Martelli, A., Ghia, M., Mereto, E., Marinari, M.U., Brambilla, G., 1988. Induction and promotion of γ-glutamyl-transpeptidase-positive foci in the rat liver by methylglyoxal. Jpn. J. Cancer Res. (Gann) 79, 666–669.

Matsuda, H., Sato, T., Nagase, H., Ose, Y, Kito, H., Sumida, K., 1992a. Aldehydes as mutagens formed by ozonation of humic substances. Sci. Total Environ. 114, 205–213.

Matsuda, H., Ose, Y., Sato, T., Nagase, H., Kito, H., Sumita, K., 1992b. Mutagenicity from ozonation of humic substances. Sci. Total Environ. 117/118, 521–529.

Matsuura, T., Owada, K., Sano, M., Saito, S., Tomita, I., Ikekawa, T., 1986. Studies on methylglyoxal. Chem. Pharm. Bull. 34, 2926–2930.

McCann, V.J., Davis, R.E., Welborn, T.A., Constable, I.J., Beale, D.G., 1981. Glyoxalase phenotypes in patients with diabetes mellitus. Aust. N.Z. J. Med. 11, 380–382.

McKie, J.H., Douglas, K.T., 1993. Structural relationships between glyoxalase I and membrane transport proteins. Biochem. Soc. Trans. 21, 540–544.

McLellan, A.C., Phillips, S.A., Thornalley, P.J., 1992. The assay of methylglyoxal in biological systems by derivatization with 1,2-diamino-4,5-dimethoxy-benzene. Anal. Biochem. 206, 17–23.

Meyerhof, O., Lohman, K., 1934. Über die enzymatische Gleichgewichtsreaktion zwischen Hexose-diphosphosaure und Dioxyacetonphosphosaure. Biochem. Zeitschr. 271, 89–110.

Meyerhof, O., 1948. New investigations on enzymatic glycolysis and phosphorylation. Experientia 4, 169–176.

Meyerhof, O., 1951. Aldolase and isomerase. In: Sumner, J.B., Myrback, K. (Eds.), The enzymes/Chemistry and Mechanism of Action, vol. II, Part 1. Academic Press, Publishers, New York, pp. 162–182.

Milligan, L.P., Baldwin, R.L., 1967. Conversion of acetoacetate to pyruvaldehyde. J. Biol. Chem. 242, 1095–1101.

Misra, K., Banerjee, A.B., Ray, S., Ray, M., 1995. Glyoxalase III from *Escherichia coli*: a single novel enzyme for the conversion of methylglyoxal into D-lactate without reduced glutathione. Biochem. J. 305, 999–1003.

Miyakawa, Y., Nishi, Y., Kato, K., Sato, H., Takahashi, M., Hayashi, Y., 1991. Initiating activity of eight parolysates of carbohydrates in a two-stage mouse skin tumorigenesis model. Carcinogenesis 12, 1169–1173.

Mizutani, T., Yoshida, K., Kawazoe, S., 1993. Formation of toxic metabolites from thiabendazole and other thiazoles in mice. Drug Metab. Disp. 22, 750–755.

Moens, H., Payne, R., Carter, N.D., Farid, N.R., 1980. Association of glyoxalase I allotypes with Graeves' disease and diabetes mellitus. Hum. Hered. 30, 62–64.

Monder, C., 1965. Oxidation of methylglyoxal to pyruvic acid by sheep-liver enzyme. Biochim. Biophys. Acta 99, 573–575.

Monder, C., 1967. Alpha-ketoaldehyde dehydrogenase, an enzyme that catalyzes the enzymatic oxidation of methylglyoxal. J. Biol. Chem. 242, 4603–4609.

Moree-Testa, P., Saint-Jalm, Y., 1981. Determination of α-dicarbonyl compounds in cigarette smoke. J. Chromatogr. 217, 197–208.

Murata, K., Tani, K., Kato, J., Chibata, I., 1980. Excretion of glutathione by methylglyoxal-resistant *Escherichia coli*. J. Gen. Microbiol. 120, 545–547.

Murata, K., Saikusa, T., Inoue, Y., Kimura, A., 1988. Molecular cloning of a gene enhancing yeast glyoxalase I activity and its effect on yeast cell size. J. Ferment. Technol. 66, 495–500.

Nagao, N., Fujita, Y., Wakabayashi, K., Nukaya, H., Kosuge, T., Sugimura, T., 1986. Mutagens in coffee and other beverages. Environ. Health Perspect. 67, 89–91.

Nagaraj, R.H., Shipanova, I., Faust, F.M., 1996. Protein cross-linking by the Maillard reaction. J. Biol. Chem. 271, 19338–19345.

Needham, J., Lehman, H., 1937. Intermediary carbohydrate metabolism in embryonic life. VIII. Glyceraldehyde and glucolysis. Biochem. J. 31, 1913–1925.

Neuberg, C., 1913. Über die Zerstörung von Milchsaurealdehyd und Methylglyoxal durch tierische Organe. Biochem. Zeitschr. 49, 502–506.

Oberley, L.W., 1988. Free radicals and diabetes. Free Rad. Biol. Med. 5, 113–124.

Oh, M.S., Phelps, K.R., Traube, M., Barbosa-Saldivar, J.L., Boxhill, C., Carroll, H.J., 1979. D-lactic acidosis in a man with the short-bowel syndrome. N. Eng. J. Med. 301, 249–252.

Ohmori, S., Mori, M., Kawase, M., Tsuboi, S., 1987a. Determination of methylglyoxal as 2-methylquinoxaline by high-performance liquid chromatography and its application to biological samples. J. Chromatogr. 414, 149–155.

Ohmori, S., Kawase, M., Mori, M., Hirota, T., 1987b. Simple and sensitive determination of methylglyoxal in biological samples by gas chromatography with electron-capture detection. J. Chromatogr. 415, 221–229.

Ohmori, S., Mori, M., Shiraha, K., Kawase, M., 1989. Biosynthesis and degradation of methylglyoxal in animals. In: Weiner, H., Flynn, T.G. (Eds.), Enzymology and Molecular Biology of Carbonyl Metabolism 2. A.R. Liss, pp. 397–412.

Okado, A., Kawasaki, Y., Hasuike, Y., Takahashi, M., Teshima, T., Fujii, J., Taniguchi, N., 1996. Induction of apoptotic cell death by methylglyoxal and 3-deoxy-glucosone in macrophage-derived cell lines. Biochem. Biophys. Res. Commun. 225, 219–224.

Osterby, R., 1986. Structural changes in the diabetic kidney. Clin. Endocrinol. Metab. 15, 733–751.

Patterson, J.W., Lazarow, A., 1955. Glyoxalase method. In: Glick, E. (Ed.), Methods of Biochemical Analysis, vol. 2. Interscience, New York, pp. 270–273.

Paulmier, C., 1986. Selenium reagents and intermediates in organic syntheses. Pergamon Press, Oxford, pp. 360–361.

Peinado, J., López-Sariano, F.J., Argiles, J.M., 1987. Gas chromatograpic method for the estimation of acetone and the metabolites in biological samples. J. Chromatogr. 415, 372–376.

Penninckx, M.J., Jaspers, C.J., Legrain, M.J., 1983. The glutathione-dependent glyoxalase pathway in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 258, 6030–6036.

Piskorska, D., Kochanski, L., 1989. Activity of erythrocytic glyoxalases I and II in bearers of certain neoplasms. Acta Med. Pol. 30, 77–81.

Pourmotabbed, T., Creighton, D.J., 1986. Substrate specificity of bovine liver formaldehyde dehydrogenase. J. Biol. Chem. 261, 14240–14244.

Principato, G.B., Talesa, V., Norton, S.J., Contelli, S., Mangiabene, C., Rosi, G., 1990. Induction of mouse liver glyoxalase I by hypobaric hypoxia. Biochem. Intern. 20, 1019–1023.

Procaccini, R.L., DeFonti, D.R., DeFeo, J.J., 1971. Inhibition of in vitro histone acetylation in a cell free rat uterine system by methylglyoxal and phenylglyoxal. Biochem. Biophys. Res. Commun. 43, 484–487.

Puskas, R., Fredd, N., Gazdar, C., Peterkofsky, A., 1983. Methylglyoxal-mediated growth inhibition in an *Escherichia coli* cAMP receptor protein mutant. Arch. Biochem. Biophys. 223, 503–513.

Racker, E., 1951. The mechanism of action of glyoxalase. J. Biol. Chem. 190, 685–696.

Racker, E., 1957. Spectophotometric enzymatic methods for the determination of aldehydes and ketoaldehydes. Methods Enzymol. 3, 293–296.

Rae, C., Berners-Price, S.J., Bulliman, B.T., Kuchel, P.W., 1990. Kinetic analysis of the human erythrocyte glyoxalase system using 1H NMR and a computer model. Eur. J. Biochem. 193, 83–90.

Rae, C., Bubb, W.A., Kuchel, P.W., 1992. Aldolase-catalyzed diketone phosphate formation from oxoaldehydes. J. Biol. Chem. 267, 9713–9717.

Ray, S., Ray, M., 1981. Isolation of methylglyoxal synthase from goat liver. J. Biol. Chem. 256, 6230–6233.

Ray, M., Halder, J., Dutta, S.K., Ray, S., 1991. Inhibition of respiration of tumor cells by methylglyoxal and protection of inhibition by lactaldehyde. Int. J. Cancer 47, 603–609.

Ray, S., Dutta, S., Halder, J., Ray, M., 1994. Inhibition of electron flow through complex I of the mitochondrial respiratory chain of Ehrlich ascites carcinoma cells by methylglyoxal. Biochem. J. 303, 69–72.

Ray, S., Bitwas, S, Ray, M., 1997. Similar nature of inhibition of mitochondrial respiration of heart tissue and malignant cells by methylglyoxal. A vital clue to understand the biochemical basis of malignancy. Mol. Cell. Biochem. 171, 95–103.

Reinke, L.A., Stohs, S.J., Rosenberg, H., 1978. Altered activity of hepatic mixed-function monooxygenase enzymes in Streptozotocin-induced diabetic rats. Xenobiotica 8, 611–618.

Riba, P., Garzó, T., Mandl, J., Kalapos, M.P., 1992. Gluconeogenesis from methylglyoxal in isolated murine hepatocytes. Does an alternative pathway exist in which pyruvate is not an intermediate? Int. J. Biochem. 24, 1721–1724.

Richard, J.P., 1985. Reaction of triosephosphate isomerase with L-glyceraldehyde 3-phosphate and triose 1,2-enediol 3-phosphate. Biochemistry 24, 949–953.

Richard, J.P., 1993. Mechanism for the formation of methylglyoxal from triosephosphates. Biochem. Soc. Trans. 21, 549–553.

Riddle, V., Lorenz, F.W., 1968. Nonenzymic, polyvalent anion-catalyzed formation of methylglyoxal as an explanation of its presence in physiological systems. J. Biol. Chem. 243, 2718–2724.

Russell, J.B., Cook, G.M., 1995. Energetics of bacterial growth: Balance of anabolic and catabolic reactions. Microbiol. Rev. 59, 48–62.

Russell, J.B., 1992. Glucose toxicity and inability of *Bacteroides ruminicola* to regulate glucose transport and utilization. Appl. Environ. Microbiol. 58, 2040–2045.

Russell, J.B., 1993. Glucose toxicity in *Prevotella ruminicola*: methylglyoxal accumulation and its effect on membrane physiology. Appl. Environ. Microbiol. 59, 2844–2850.

Salem, H.M., 1954. Glyoxalase and methylglyoxal in thiamine-deficient rats. Biochem. J. 57, 227–230.

Sarkar, S., Bhaduri, A., 1986. Anti-leishmanial activity of methylglyoxal. Indian J. Exp. Biol. 24, 296–298.

Schauestein, E., Esterbauer, H., Zollner, H., 1980. α-Dicarbonyls in: Aldehydes in biological systems. Pion Press, London, pp. 112–157.

Shamsi, F.A., Partal, A., Sandy, C., Glomb, M.A., Nagaraj, R.H., 1998. Immunological evidence for methylglyoxal-derived modifications in vivo. J. Biol. Chem. 273, 6928–6936.

Shinohara, M., Giardino, I., Brownlee, M., 1996. Overexpression of glyoxalase I inhibits intracellular advanced glycation end-product. Diabetes 45 (suppl. 2), 126A.

Simons, J.O., Snoep, J.L., Feitz, S., Teixeira de Mattos, M.J., Neijssel, O.M., 1992. Anaerobic 2-ketogluconate metabolism of *Klebsiella pneumoniae* NCTC 418 grown in chemostat culture: involvement of the pentose phosphate pathway. J. Gen. Microbiol. 138, 423–428.

Sjolemma, B., Seekles, L., 1926. Über die Zuckerbildung aus dem methylglyoxal in normalen Tierkörper. Biochem. Zeitschr. 176, 431–440.

Speck, J.C., 1958. The Lobry de Bruyn-Alberda van Ekenstein transformation. Adv. Carbohydr. Chem. 56, 63–103.

Spoehr, H.A., Milner, H.W., 1934. Studies on atmospheric oxidation. III. The catalytic oxidation of trioses and related compounds. J. Am. Chem. Soc. 56, 2068–2074.

Steinberg, S., Kaplan, I.R., 1984. The determination of low molecular weight aldehydes in rain, fog and mist by reversed phase liqoid chromatography of 2,4-dinitrophenylhydrazone derivatives. Intern. J. Environ. Anal. Chem. 18, 253–266.

Strzinek, R.A., Vela, G.R., Scholes, V.E., Norton, S.J., 1970. Further studies on liver glyoxalase activity in mice bearing lymphosarcoma. Cancer Res. 30, 334–337.

Sueoka, T., Katoh, S., 1995. Carbonyl reductase activity of sepiapterin reductase from rat erythrocytes. Biochim. Biophys. Acta 843, 193–198.

Sugimura, T., Sato, S., 1983. Mutagens-carcinogens in foods. Cancer Res. 43, 2415s–2421s.

Szent-Györgyi, A., McLaughlin, J.A., 1975. Interaction of glyoxal and methylglyoxal with biogenic amines. Proc. Natl. Acad. Sci. USA 72, 1610–1611.

Szent-Györgyi, A., 1965. Cell division and cancer. Science (Washington D.C.) 149, 34–37.

Szent-Györgyi, A., 1973. Biolelectronics and cancer. Bioenergetics 4, 533–562.

Takahashi, M., Okamiya, H., Furukawa, F., Toyoda, K., Sato, H., Imaida, K., Hayashi, Y., 1989. Effects of glyoxal and methylglyoxal administration on gastric carcinogenesis in Wistar rats after initiation with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Carcinogenesis 10, 1925–1927.

Takayama, K., Nakano, M., Zinner, K., Vidigal, C.C.C., Durán, N., Shimizu, Y., Cilento, G., 1976. Generation of electronic energy in the myoglobin-catalyzed oxidation of acetoacetate to methylglyoxal. Arch. Biochem. Biophys. 176, 663–670.

Taylor, D.G., Trudgill, P.W., Cripps, R.E., Harris, P.R., 1980. The microbial metabolism of acetone. J. Gen. Microbiol. 118, 159–170.

Thornalley, P.J., Wolff, S., Crabbe, J., Stern, A., 1984. The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalysed by buffer ions. Biochim. Biophys. Acta 797, 276–287.

Thornalley, P.J., 1985. Monosaccharide autoxidation in health and disease. Environ. Health Perspect. 64, 297–307.

Thornalley, P.J., 1993. The glyoxalase system in health and disease. Mol. Aspects Med. 14, 287–371.

Thornalley, P.J., 1994. Methylglyoxal, glyoxalases and the development of diabetic complications. Amino Acids 6, 15–23.

Tokunaga, K., Omoto, K., Juji, T., Itoh, T., 1982. Polymorphism of BF, C2, and GLO in Japanese patients with insulin-dependent diabetes mellitus: Confirmation of an increase of BFαFT. Hum. Genet. 62, 86–88.

Tötemeyer, S., Booth, N.A., Nichols, W.W., Dunbar, B., Booth, I.R., 1998. From famine to feast: the role of methylglyoxal production in *Escherichia coli*. Mol. Microbiol. 27, 553–562.

Uchida, K., Khor, O.T., Oya, T., Osawa, T., Yasuda, Y., Miyata, T., 1997. Protein modification by a Maillard reaction intermediate methylglyoxal. FEBS Lett. 410, 313–318.

Uotilla, L., Koivusalo, M., 1974. Formaldehyde dehydrogenase from human liver. J. Biol. Chem. 249, 7653–7663.

Urata, G., Granick, S., 1963. Biosynthesis of α-aminoketones and the metabolism of aminoacetone. J. Biol. Chem. 238, 811–820.

Valentine, W., Paglia, D.E., Neerhout, R.C., Konrad, P.N., 1970. Erythrocyte glyoxalase II deficiency with coincidental hereditary elliptocytosis. Blood 36, 797–808.

Vander Jagt, D.L., Hunsaker, L.A., Robinson, B., Stangebye, L.A., Deck, L.M., 1990a. Aldehyde and aldose reductases from human placenta. J. Biol. Chem. 265, 10912–10918.

Vander Jagt, D.L., Hunsaker, L.A., Campos, N.M., Baack, B.R., 1990b. D-lactate production in erythrocytes infected with *Plasmodium falciparum*. Mol. Biochem. Parasitol. 42, 277–284.

Vander Jagt, D.L., Robinson, B., Taylor, K.K., Hunsaker, L., 1992. Reduction of trioses by NADPH-dependent aldo-keto-reductases. J. Biol. Chem. 267, 4364–4369.

Vander Jagt, D.L., 1982. 2-ketoaldehyde dehydrogenase from rat liver. Methods Enzymol. 89, 513–515.

Vander Jagt, D.L., 1989. The glyoxalase system. In: Dolphin, D., Poulson, R., Avramovic, O. (Eds.), Glutathione: Chemical, Biochemical and Medical Aspects — part A. New York, John Wiley and Sons, pp. 597–641.

Vander Jagt, D.L., 1993. Glyoxalase II: Molecular characteristics, kinetics and mechanism. Biochem. Soc. Trans. 21, 522–526.

Vidigal, C.C.C., Cilento, G., 1975. Evidence for the generation of excited methylglyoxal in the myoglobin catalyzed oxidation of acetoacetate. Biochem. Biophys. Res. Commun. 62, 184–190.

Vince, R., Wadd, W.B., 1969. Glyoxalase inhibitors as potential anticancer agents. Biochem. Biophys. Res. Commun. 35, 593–598.

Vogt-Moller, P., 1929. Ist Avitaminosis B1 eine Intoxikation mit Methylglyoxal? Biochem. Zeitschr. 233, 248–250.

von Pechmann, H., 1887. Über die Spaltung der Nitrosoketone. Ber. Dtsch. Chem. Gesells. 20, 3213–3214.

Wermuth, B., 1981. Purification and properties of an NADPH-dependent carbonyl reductase from human brain. J. Biol. Chem. 256, 1206–1213.

Winter, R., Piskorska, D., Jerzykowski, J., 1978. Further studies on liver glyoxalase I and glyoxalase II. Activity in mice bearing sarcoma 180 and L1210 leukemia. Neoplasma 25, 465–470.

IARC Working Group on the evaluation of carcinogenic risks

to humans, 1991. Methylglyoxal. In: Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 51. IARC, Lyon, pp. 443–458.
Yamaguchi, T., Nakagawa, K., 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agric. Biol. Chem. 47, 2461–2465.
Yim, H.S., Kang, S.O., Hah, Y.C., Chock, P.B., Yim, M.B., 1995. Free radicals generated during the glycation reaction of amino acids by methylglyoxal. J. Biol. Chem. 270, 28228–28233.
Yu, P.H., 1998. Deamination of methylamine and angiopathy: toxicity of formaldehyde, oxidative stress and relevance to protein glycoxidation in diabetes. J. Neural. Transm. 52, s201–s216.